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GENETIC FACTORS FOR PIGMENTATION IN THE ONION AND THEIR RELATION TO DISEASE RESISTANCE[1]

By G. H. RIEMAN[2]

Assistant Geneticist, Department of Genetics, Agricultural Experiment Station, University of Wisconsin, and Agent, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The common onion (*Allium cepa* Linn.) has been used extensively in studies on the nature of disease resistance. It has been demonstrated that, in general, pigmented onion bulbs are highly resistant to the onion smudge organism (*Colletotrichum circinans* (Berk.) Vogl.) while pigmentless bulbs are susceptible. This relationship between parasite and host affords a rather unique opportunity for fundamental research on the nature and inheritance of disease resistance. Although many cases of disease resistance in plants have been reported, in no other instance, so far as the writer is aware, has a specific visible character been recognized which is definitely associated with a lethal effect on the parasite. Not only are the pigments located in the outer scales of the bulb known to be associated with toxic materials, but a toxic chemical entity has been isolated from pigmented scales that is not present in pigmentless scales (*5*, *12*).[3]

Tschermak (*8*) reports that dark yellow and red bulb colors are dominant to white in the F_1 generation. In the F_2 generation a complex segregation was observed, and in the F_3 generation some white individuals produced white and colored bulbs. He observed that the so-called white commercial varieties of onions produce many plants showing light-red or yellowish pigmentation. The data presented in this paper also show that the yellow and red bulb colors are dominant to a recessive white in the F_1 and later generations. However, a dominant white was also noted, which readily accounts for the complex segregation Tschermak encountered in the F_2 and F_3 generations resulting from open-pollinated insect crosses.

The nature of the interaction of color factors which determine the physiological expression of chemical entities responsible for resistance found in the outer scales of the onion form the basis for this study.

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[3] Reference is made by number (italic) to Literature Cited, p. 277.

DESCRIPTION OF THE SMUDGE DISEASE

Smudge occurs on the common onion, shallots, and leeks, causing a marred appearance, shrinkage of bulbs, and premature sprouting. The disease may develop at any time during the life of the plant. The most common symptom is the dark-green or black smudge on the bulb or neck of the onion. (Pls. 1 and 2.) In some cases the surface of the lesion may be uniformly black, but more commonly the stromata and visible mycelium of the parasite are arranged in concentric circles. In severe cases the fungus grows through the outer dry scales into the turgid living tissue, causing a collapse of the fleshy scales. On pigmented onions the fungus is confined almost entirely to the neck of the bulb, where the flattened leaves are colorless.

RELATION OF THE PARASITE TO SCALE PIGMENTATION

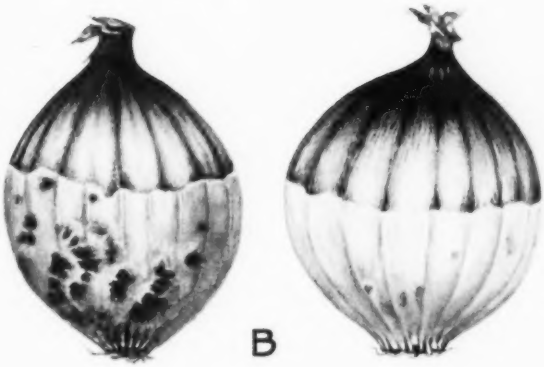
In reports (*9*, *10*, *11*) dealing with the parasitism of the smudge organism it has been shown that the host tissues contain two classes of toxic substances. One of these was found in the cell sap, the other was associated with pigmentation in the dried outer scales. It was found that water extracts from the outer dry colored scales contained toxic substances which, in sufficient concentration, either entirely prevented spore germination or caused abnormal germ-tube development. As the concentration of the extract was reduced, the toxic effects were proportionally decreased until eventually normal germination and growth occurred. Water extracts from the outer dried white scales did not inhibit spore germination or germ-tube development. It was concluded, therefore, that the resistance of colored onions to the smudge disease was due to a substance or substances either closely associated or identical with the red and yellow pigments present in the outer scales. It was suggested that these water-soluble toxins of the host were located in an advantageous position to serve as a barrier to the invasion of the parasite. In nature these toxic substances might readily dissolve into the moisture which comes in contact with the pigmented scales, producing a natural fungicidal effect on the parasite. It has been demonstrated (*6*), that pigmented scales which had been badly bleached by the action of meteoric or soil water contain less toxin than scales in which pigment preservation is at a maximum. In recent studies (*5*) a toxic entity, protocatechuic acid, was isolated from colored scales which was not present in white scales. This phenolic acid was found in a free state in colored outer scales. In dilutions of 1 to about 3,000 its toxicity resembled that of the crude water extract from which it was isolated.

NATURE OF THE MATERIAL AND EXPERIMENTAL PROCEDURE

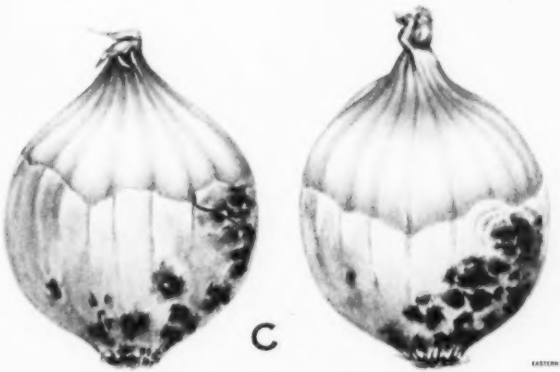
Although varieties of common onions vary somewhat in intensity of color, they can be roughly grouped into red, yellow, and white classes. Many varieties, like St. Marie Rose and Australian Brown, lie on the border line between three groups. The pigments of onion scales are solutes in the cell sap of the outer epidermal layer. A dilute color first appears when the plants are about half grown. The intensity increases during development and at maturity pigmentation is at a maximum in the dried outer scales. Epidermal

Typical susceptible white onion bulbs infected with smudge are pictured at the top (A). Note the black smudged appearance of the bulb on the right and the characteristic concentric circles of mycelium on the bulb on the left. Resistant red (B) and yellow (C) bulbs are shown below

A



B



C

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Typical red bulbs (A), red neck bulbs (B), and white bulbs (C), after the dried outer scales have been removed from the upper portions. Note the splashes of red on the fleshy scales of red neck bulbs (B) in contrast to the greenish white appearance of the fleshy scales of the white bulbs (C). Onion smudge appears on three of the lower four bulbs

cells of yellow varieties turn a deep brownish-yellow when treated with alkalies, a reaction typical of flavones (7). Epidermal cells of red varieties turn pink in acid and green in alkaline solutions, which are characteristic reactions of anthocyans.

In nature the common onion is a highly cross-pollinated plant. Self-pollination usually results in a loss of general plant vigor and in forms of sterility, the nature of which has not been determined. A few fertile inbred lines have been obtained which exhibited a fair amount of vigor after three generations of self-pollination. Considerable embryo degeneration occurred in both cross-pollinated and self-pollinated lines.

Crosses were made in the field and in the greenhouse. The umbels were allowed to develop normally until a few flowers opened and then the opened and the small immature flowers were plucked. The inflorescences were washed with water before the flowers were emasculated. Finely pointed tweezers were used to clip off the top portions of the perianth segments and to remove the anthers. From 40 to 100 flowers were worked on each umbel. Cross-pollinations were effected by rubbing dehiscing anthers on the stigmas of emasculated flowers. Self-pollinations were made by covering the umbels before the flowers opened. All pollinating was carefully controlled by covering the inflorescences with manila or vegetable parchment bags.

Since segregation was observed in the F_1 progenies of reciprocal crosses between red and white onions, tests were made to determine whether or not apogamous development had occurred. From 10 to 15 flowers in 17 different umbels were emasculated in the usual manner, but no pollen was applied to the stigmas. Some of these unfertilized ovaries showed parthenocarpic development up to about one-half the normal size of ripening fertilized ovaries but then degenerated. These tests also demonstrated the thoroughness of the crossing technic employed.

Approximately 50 per cent of the various crosses attempted were successful. The usefulness of many of the crosses obtained was seriously impaired because (1) many or all the plants failed to set seed in later generations, (2) seed bulbs were lost as a result of storage rots, and (3) seedling progenies were destroyed by damping-off fungi.

Sporulating cultures of *Colletotrichum circinans* grown on potato-dextrose agar were used to make up spore suspensions of the smudge organism. Bulbs growing in the field during 1924 were inoculated with the spore suspension by means of a hand-pressure spray. Conditions were favorable for the development of the disease, and with few exceptions all the white bulbs showed typical smudge lesions. With the exception of relatively inconspicuous lesions on the unpigmented neck portions of some of the colored bulbs, all the red and yellow onions appeared to be immune. Sporulating cultures were not available for inoculating the 1925 crop, so diseased scales from onions infected with smudge were macerated with sand and applied along with mascerated mycelial cultures. Weather conditions were unfavorable for the development of the disease; consequently resistance records could not be obtained. Two field inoculations of mixtures of spore suspension and macerated mycelium from pure cultures were made during the summer of 1926. Warm weather and frequent rains following the inoculations favored disease development. Typical smudge lesions were prevalent at harvest time. A fairly high

percentage of disease-free white bulbs were observed. During 1927 two spore-suspension inoculations were made in the field. Smudge symptoms developed rapidly, and at harvest time many bulbs were badly diseased. Although care was taken to distribute the inoculum evenly, and two applications at intervals of two weeks were made to insure uniform infestation, an uneven distribution of the disease on white bulbs was noted at harvest time. In general the plants which developed the major portion of the bulb above the soil line were less diseased than those which had grown deeper in the soil. Low and poorly drained areas of the plot contained a larger precentage of infected white bulbs than well-drained areas. These results indicated that the methods of field inoculation employed offered a very crude measure of disease resistance.

Tests employed earlier by another investigator (*10*) were used to demonstrate variation in the amount of toxic materials in the outer scales of the white bulbs of F_2 and F_3 progenies resulting from reciprocal crosses of red and white onions. The method used was as follows: Drops of distilled water containing spores of the fungus were placed on clean glass slides, two drops on each slide, and disks of onion scales about 6 millimeters in diameter, made with a paper punch, were placed in the drops, usually two such pieces in each drop. The slides were then put in Petri dishes lined with moistened filter paper which served as moist chambers. Observations on the amount and character of spore germination were made and recorded after 18 to 48 hours.

Probable errors and goodness of fit were determined wherever necessary. The probable errors of Mendelian ratios were calculated according to the formula $0.6745 \sqrt{p \times q \times n}$, where n is the total number of individuals in the population, p and q are the two elements in a ratio, as 0.75 and 0.25, 0.5 and 0.5, 0.81 and 0.19, whose sums total 1.000. The probable errors thus obtained were compared with the deviations of the actual numbers from those expected on the basis of the given ratio. For tests of goodness of fit in cases where the number of classes was more than two, use was made of the χ^2 method as suggested by Harris (*1*).

WORKING HYPOTHESES FOR COLOR INHERITANCE

Five different genes are necessary to account for the genetic behavior of the four bulb color classes—red, yellow, red neck, and white—observed in the onion stocks dealt with in this paper. (Pls. 1 and 2.) These five genes are given the following symbols:

(1) *I*—a gene for incomplete inhibition of color.
(2) *i*—a gene allowing expression of color.
(3) *W*—a gene for red pigment.
(4) W^y—a gene for yellow pigment.
(5) *w*—a gene for white.

The genes *W*, W^y, and *w* are considered multiple allelomorphs showing independent inheritance of the genes *I* and *i*, which constitute a factor pair. The members of the allelomorphic series and the independent factor pair exhibit the following relations:

I is incompletely dominant to *i*.
W is dominant to *w*.
W is dominant to W^y.

The relationship between the gene W^y and the gene w is not clearly understood.

The phenotypic formulae assigned to the four bulb color classes under consideration are as follows:

(1) *Wi*—red.
(2) W^yi—yellow.
(3) *WIi*—red neck.
(4) *WII*, *wI*, *wi*—white.

From an examination of the factorial assignments listed above it is evident that the various bulb color classes include the following genotypes: Red, *WWii*, *Wwii*, and WW^yii; yellow, W^yW^yii and W^ywii; red neck, *WWIi* and *WwIi*; white, *WWII*, *WwII*, *wwII*, *wwIi*, and *wwii*.

EXPERIMENTAL REPORT

All the material pertaining to the genetic relations of red and white classes is considered first. This includes the red-neck class found among genetically white plants. Next, the genetic relations of the red, yellow, and white classes are considered. Applicable data from all four color classes are then used to indicate the relation of the color factors to disease resistance.

RED AND WHITE COLOR RELATIONS

Reciprocal crosses were made between red and white open-pollinated stocks of onions. The red parent selfed resulted in 14 red bulbs, showing that it was probably homozygous for factors controlling the production of red pigment. Attempts to self the white parent failed. Red and white bulbs appeared in the F_1 of the red and white crosses. (Table 1.) This at once indicated that the white parent was probably heterozygous for factors controlling pigment production.

Attempts were made to self all the F_1 plants, but because of poor bulb development and storage rots, F_2 and F_3 progenies were obtained from only six F_1 individuals. Fortunately, of the six, three were white and three were red. Each of the three F_1 white bulbs was also crossed with a different F_1 red bulb.

The F_1, F_2, and F_3 data from reciprocal crosses No. 2 and No. 3 indicate that two factor pairs control the expression and production of red pigment. The *Ii* factor pair involving inhibition of color was found to be heterozygous in the open-pollinated P_1 white bulb. The other factor pair governing the production of red pigment proved to be homozygous dominant in the P_1 red bulb and homozygous recessive in the P_1 white bulb.

The parental genotypic form ulae involved may be given as follows:

Cross No. 2 (Red×White), *WWii*×*wwIi*.
Cross No. 3 (White×Red), *wwIi*×*WWii*.

Under such circumstances the red F_1 bulbs should have the genetic formula *Wwii* and the white F_1 bulbs should have the genetic formula *WwIi*, and they should occur in equal numbers.

TABLE 1.—*F_1 data from reciprocal crosses between red and white onion bulbs*

Cross No.	Pedigree numbers	Parental genotypes	F_1 progenies		Deviation	Dev. / P. E.
			Red	White		
2	$R_{11} \times W_{22}$	*WWii*×*wwIi*	9	7	1.0±1.4	0.7
3	$W_{22} \times R_{11}$	*wwIi*×*WWii*	9	3	3.0±1.2	2.5
Total			18	10	4.0±1.8	2.2
Theoretical, 1:1			14	14		

When the three remaining white F_1 plants were self-fertilized they exhibited distinct 3:13 ratios of red and white bulbs. The three red F_1 plants yielded 3:1 ratios of red and white bulbs. The results of these F_2 progenies are presented in Table 2.

TABLE 2.—*F_2 data from reciprocal crosses between red and white onion bulbs*

F_1 bulb No.	F_1 genotypic formulae	F_2 progenies		Deviation	Dev. / P. E.	F_1 bulb No.	F_1 genotypic formulae	F_2 progenies		Deviation	Dev. / P.E.
		Red	White					Red	White		
2-1	*Wwli*	31	137	0.5±3.4	0.1	2-4	*Wwii*	50	20	2.5±2.4	1.0
2-2	*Wwli*	28	143	4.1±3.4	1.2	2-5	*Wwii*	67	38	11.8±3.0	3.9
3-1	*Wwli*	32	166	5.1±3.7	1.4	3-2	*Wwii*	15	4	.8±1.3	.6
Total		91	446	9.7±6.1	1.6	Total		132	62	13.5±4.1	3.3
Theoretical, 3:13		100.7	436.3			Theoretical, 3:1		145.5	48.5		

A further test of the validity of the hypothesis offered is afforded by the results of progeny tests in generations beyond the F_2. Nine F_2 genotypes showing the breeding behavior indicated are called for, as follows:

1 *WWII*—all white.
2 *WwII*—all white.
2 *WWIi*—1 red : 3 white.
4 *WwIi*—3 red1 : 3 white.
1 *wwII*—all white.
2 *wwIi*—all white.
1 *WWii*—all red.
2 *Wwii*—3 red : 1 white.
1 *wwii*—all white.

A total of 105 white F_2 plants were selfed and progeny tests were conducted on a relatively large scale. Progenies from selfed white F_2 plants are arranged in Table 3.

TABLE 3.—*F_3 data from selfed white F_2 plants of onion progenies segregating for red and white in 3:13 ratios*

Parent bulbs having genotypic formulae *WwIi* or *WWIi*							Parent bulbs having genotypic formulae *WWII, WwII, wwII, wwIi, wwii*	
F_2 bulb No.	F_3 progenies		Ratio for 24 individuals		Deviation in red class		F_2 bulb No.	All white F_3 progenies
	Red	White	Red	White	−	+		
46-2	8	44	3.7	20.3	1.3		48-1	17
46-4[a]	3	7					48-2	81
47-1	2	16	2.7	21.3	2.3		48-4	20
47-3	14	19	10.2	13.8		5.2	49-7	47
47-4[a]	1	9					52-1	374
47-5	4	24	3.4	20.6	1.6		53-5[a]	11
49-2	10	24	7.0	17.0		2.0	54-3	326
51-1	79	323	4.7	19.3	.3		54-7	94
51-3	2	14	3.0	21.0	2.0		54-9	39
51-4	6	25	4.6	19.4	.4		54-10	139
52-2	12	57	4.2	19.8	.8		54-12	44
52-3[a]	1	6					54-13	49
52-4	11	33	6.0	18.0		1.0	54-14[a]	14
53-2	11	26	7.1	16.9		2.1	54-15	107
53-4	4	27	3.1	20.9	1.9		54-16	48
53-7	3	19	3.3	20.7	1.7		55-1[a]	13
53-8	2	17	2.5	21.5	2.5		55-2[a]	3
53-9	9	30	5.5	18.5		.5	55-4[a]	9
53-10	142	626	4.4	19.6	.6		55-6	49
54-4	23	80	5.4	18.6		.4	55-8	26
54-17	3	16	3.8	20.2	1.2		55-9	231
54-18	4	33	2.6	21.4	2.4		55-11[a]	3
54-19	22	49	7.4	16.6		2.4	55-12	20
54-20[a]	1	2					55-13	504
55-5	17	39	7.3	16.7		2.3	55-18	132
55-10	7	49	3.0	21.0	2.0		55-19	129
55-15	34	155	4.3	19.7	.7		55-20	73
55-17[a]	3	12					55-22	30
11b-1[a]	6	7					55-23	107
11c-1	96	334	5.4	18.6		.4	55-26	184
57-4	71	218	5.9	18.1		.9	55-27	29
57-2[a]	1	5					55-28[a]	11
57-5	12	120	2.2	21.8	2.8		55-30	105
58-1	45	352	2.7	21.3	2.3		55-33	21
58-2	14	48	5.4	18.6		.4	56-34	20
58-3	5	31	3.3	20.7	1.7		59-1	171
58-4	4	26	3.2	20.8	1.8		59-2[a]	11
58-55	4	36	2.4	21.6	2.6		59-5	46
58-7	11	20	8.5	15.5		3.5	59-7	60
59-1	57	217	5.0	19.0	0	0	59-12	25
59-3	22	91	4.7	19.3	.3		59-14	78
59-4	12	106	2.4	21.6	2.6		59-16	28
59-6	18	139	2.8	21.2	2.2		59-19	32
59-11	20	113	3.6	20.4	1.4		59-20[a]	4
59-13	14	85	3.4	20.6	1.6		60a-2	40
60a-1	3	36	1.8	22.2	3.2		60a-6	43
60a-16	62	302	4.1	19.9	.9		60a-7	24
60a-21a	1	9					60a-14	22
60b-1	4	16	4.8	19.2	.2		60a-20	83
60b-3	22	92	4.6	19.4	.4			
60b-5	10	29	6.2	17.8		1.2		
60b-7	23	154	3.1	20.9	1.9			
60b-8	4	25	3.3	20.7	1.7			
60b-9	7	58	2.6	21.4	2.4			
60b-12	35	130	5.1	18.9		.1		
60b-15	4	18	4.4	19.6	.6			
Total	[a] 1,008	[a] 4,541						[a] 3,727
Total number of progenies					33	14		
Theoretical, 2:1					31.3	15.7		
Deviation					1.7±1.9			
Dev.								
P. E.					1.1			
Total number of progenies					48			40
Theoretical, 6:7					40.6			47.4
Deviation					7.4±3.2			
Dev.								
P. E.					2.3			

[a] Progenies containing less than 16 individuals are not considered because of the uncertainty in distinguishing true breeding from segregating families.

The F_3 data from selfed white F_2 plants of progenies segregating for red and white in 3:13 ratios show that the total number of segregating families and true-breeding white families agrees with the expected 6:7 ratio. With the number of individuals available it is not possible to differentiate definitely between the two types of ratios, 1:3 and 3:13, expected among the segregating progenies because their numerical proportions are quite similar. Critical progeny tests of some of the segregating F_3 families will be presented later. At this point it is of interest to know whether the ratios obtained fall into the expected narrow range. Therefore, the two types of ratios were combined in the proportion in which they would be expected to occur. Thus, the 1:3 ratio was doubled and added to the 3:13 ratio, resulting in a ratio of 5:19. This combined ratio allows for the occurrence of twice as many plants having the genetic formula *WwIi* as those having the genetic formula *WWIi*. In order to offset to a certain degree the influence of variation in numbers of individuals within families which range between 16 and 768, the actual ratio was weighted in terms of 24 individuals. Examination of the individual entries in Table 3 shows that, in general, the deviations are not excessively large. On the basis of chance the plus and minus deviations for the 48 families should approximate a 2:1 ratio. The 33 minus deviations and the 14 plus deviations found in the red class are in accord with the hypothesis presented in this paper.

PHENOTYPIC EXPRESSIONS OF THE INTERACTION OF THE COLOR FACTORS

It is of interest to demonstrate that 1:3 and 3:13 ratios of red and white are present in the segregating F_3 progenies. One line of evidence is presented by the interaction of the *Ww* factor pair with the *Ii* factor pair.

Cream-colored bulbs of varying intensity have been observed in fairly large numbers in all progenies possessing the *W* gene and segregating for the *I* gene. Only an occasional light cream-colored bulb has been noted in progenies breeding true for white. Another condition, called red neck, has also been observed frequently on genetically white bulbs in most of the progenies carrying the *W* gene and segregating for the *I* gene. These observations indicate that the two conditions, cream and red neck, are similar in nature and that they are the phenotypic expression of the interaction of the heterozygous *Ii* factor pair and the gene *W*. Red neck usually appears as narrow, red areas on the surface of the inner fleshy scales in the neck region of the bulbs. (Pl. 2.) It varies in intensity from almost full red to minute red areas that can be seen only with the aid of a hand lens. In nearly all cases bulbs having red neck were cream colored, though many cream-colored bulbs do not show red neck.

A classification of the segregating and the true white-breeding F_3 progenies shows that in only 1 case out of 40 were true-breeding white F_2 bulbs classified as cream. Fifteen segregating F_2 bulbs out of 46 were classified as white. These relations clearly show that the cream character is closely associated with the heterozygous condition of the *Ii* factor pair in the presence of the *W* gene.

Since the cream character was difficult to classify and a close correlation was noted between it and the red-neck character it was considered advisable to classify the F_3 bulbs according to the presence of red color on the inner fleshy scales. Obviously the expression of a

delicate color character protected by plant tissues should be more constant than one expressed in external plant tissues which are exposed to weathering conditions. Then, too, the outer scale of the bulb is often lost in harvesting, which might cause a cream bulb to appear white.

The available data on the occurrence of these intermediate characters associated with the heterozygous condition of the *I* gene in the presence of the *W* gene are presented in Table 4. Many of the bulbs could not be classified because of field and storage rots or poor bulb development.

TABLE 4.—*Occurrence of white and cream and red-neck colored F_3 onion bulbs*

F_2 bulb No.	F_3 progenies segregating for genetically red and white bulbs		F_2 bulb No.	F_3 progenies breeding true for genetically white bulbs	
	White and cream	Red neck		Pure white	White and cream
46-2	26		48-1	10	
46-4	2	5	48-2	33	
47-1	11		48-4	17	
47-3	11	8	52-1	76	
47-5	22	1	54-3		123
49-2	8		54-7		94
51-4	4		54-10	139	
52-2	18	25	54-12	35	
52-4	11	0	54-13		9
53-2	19	7	54-15		[a]107
53-7	6		55-6		13
53-8	11	6	55-13		[a] 498
53-10	247	284	55-18	106	
54-4[b]	62		55-19	97	
54-19[b]	40		55-20		17
55-5	8	1	55-23		103
55-10	17		55-26	37	
55-15	49		55-27	9	
55-17	3		55-30	63	
57-4	72	113	55-34	23	
57-5	64	53	56-1		33
58-1	157	93	59-5	42	
58-2	19	20	59-7	50	
58-3	9	14	59-12		16
58-4	4	5	59-19		21
58-5	27	6	60a-2	22	
58-7	12	7	60a-6		25
59-1	148	60	60a-7		19
59-3	56	32	60a-14		11
59-4	45	39	60a-20		74
59-6	85	48			
59-11	38	20			
59-13	60	19			
60a-1	44				
60a-16	12	8			
60b-3	43	28			
60b-5	6	18			
60b-7	75	71			
60b-8	13	12			
60b-9	45	8			
60b-12	19	26			
60b-15	8	3			
Total number of progenies	42	32	Total number of progenies	15	15

[a] An occasional red-neck bulb was observed.

[b] Red-neck bulbs present.

The data in Table 4 show that the red-neck character is associated with progenies segregating for genetically red and white bulbs, indicating that its expression depends upon the heterozygous condition of the *Ii* factor pair in the presence of the *W* gene. In only

2 of the 30 families breeding true for genetically white bulbs did an occasional red-neck bulb appear. The occurrence of a few red-neck bulbs in families breeding true for genetically white bulbs is not surprising if this character is dependent upon a heterozygous condition. The expression of color in full red bulbs does not take place until the plants are about half grown, showing that color differentiation is dependent upon physiological changes occurring late in the development of the plant. Likewise the red-neck character is most strongly expressed in well-matured bulbs.

Table 5 shows that in the F_3 progeny No. 262, segregating for red and white, all the genetically white, red-neck bulbs carried the *W* gene and were heterozygous for the *I* gene and all the pure white bulbs lacking the red-neck character possessed one of the five possible genotypes as follows: *WWII*, *WwII*, *wwII*, *wwIi*, and *wwii*.

TABLE 5.—*Breeding behavior of pure white and red-neck F_3 onion plants*

F_3 bulb No.	F_3 phenotype	F_4 progenies		F_3 bulb No.	F_3 phenotype	F_4 progenies	
		Red	White			Red	White
262W–2a	Pure white	0	20	262Rn–1a	Red neck	3	19
262W–3a		0	8	262Rn–1		3	22
262W–3		0	17	262Rn–2		5	18
262W–4a		0	16	262Rn–3		2	17
262W–5a		0	15	262Rn–4		12	38
262W–7		0	14	262Rn–5		8	48
262W–9		0	8	262Rn–6		7	32
262W–10		0	23	262Rn–7		2	31
262W–14a		0	43	262Rn–8		8	31
262W–15a		0	7	262Rn–9a		4	13
262W–20a		0	108	262Rn–9		0	13
				262Rn–10		6	38
				262Rn–17		2	19
				262Rn–18a		0	4
				262Rn–19a		6	18
Total		0	279	Total		68	361

Attempts were made to test out the five genotypically different whites occurring in the 3 red : 13 white ratio. The types of progenies resulting from crosses between the five different whites and a true-breeding red should be as follows:

P_1 genotypes	F_1 phenotypes
1 *WWII*×*WWii*	All white.
2 *WwII*×*WWii*	Do.
1 *wwII*×*WWii*	Do.
2 *wwIi*×*WWii*	1 white : 1 red.
1 *wwii*×*WWii*	All red.

Ten crosses between true-breeding white and red plants are presented in Table 6. Red plants from an all red F_3 progeny of 138 individuals resulting from a selfed red F_2 plant were used for the red parents. Six different white F_2 plants which gave all white F_3 progenies were used for the white parents. All but one of the red parents used in the crosses were selfed and found to be homozygous red.

TABLE 6.—*F_1 data from crosses between true-breeding white and red onion plants*

Cross No.	Pedigree Nos.	F_1 progenies		Cross No.	Pedigree Nos.	F_1 progenies	
		Red	White			Red	White
45	54-12×181-11	0	16	52	181-3×60a-14	0	1
46	181-11×54-12	0	17	53	181-16×60a-14	3	4
47	181-7×55-18	0	27	54	181-18×60a-14	1	0
48	55-18×181-6	0	19				
49	55-19×181-11	0	12				
50	55-20×181-11	0	4				
51	181-15×55-30	0	23				
Total		0	118	Total		4	5
Expected		0	118	Expected, 1:1		4.5	4.5

Of the six different whites used in the crosses five gave all white plants and one gave red and white plants in the ratio of 1:1. On the assumed theoretical basis there are four chances in seven of obtaining white plants that would give all white offspring when crossed with homozygous red plants and two chances in seven of obtaining white plants that would give a ratio of one red to one white when crossed with homozygous red plants. The results of the crosses listed in Table 6 agree very closely with the expected. Five out of six white plants used in crosses with red plants gave all white offspring and only one out of six gave a 1:1 ratio of red and white. These results indicate the presence of white plants with the genotypic formula *wwIi* in progenies segregating for 3:13 ratios of red and white. They also give some evidence of the occurrence of the three expected genotypes *WWII*, *WwII*, and *wwII*.

The distribution of the red-neck character indicates strongly that genetically white plants having the genotypic formulae *WWIi* or *WwIi* are present in progenies segregating for red and white. It has been shown in Table 5 that red-neck bulbs are heterozygous for the *Ii* factor pair. Therefore, in a consideration of only the white and red-neck classes, a selfed white plant having the genotypic formula *WwIi* should give six red-neck plants to seven white or cream plants, and a selfed white plant having the genotypic formula *WWIi* should give two red-neck plants to one white plant. Even though the expression of the heterozygous character red neck is not constant, the numerical difference between a 6 : 7 and a 2 : 1 ratio should be sufficiently large to enable one to distinguish between these two groups.

If the segregating progenies having a larger number in the red-neck class than in the white and cream class are grouped (Table 7) it becomes apparent that a grouping of certain 1 : 3 ratios has been effected. The 1 red : 3 white ratio fits the total population better than the 3 red : 13 white ratio. The two progenies 57–4 and 60b–5, which show fairly close agreement with a 2 : 1 ratio of cream red-neck bulbs to white and cream bulbs (Table 4), also show a close fit to the 1 : 3 ratio of red and white $\left(\frac{\text{Dev.}}{\text{P. E.}} = 0.3 \text{ and } 0.2, \text{ respectively}\right)$ (Table 3). Furthermore, progeny tests of these two families, which are recorded in Table 8, show that the red individuals were all homozygous, as would be expected in the 1 red : 3 white ratios.

TABLE 7.—*F_3 onion progenies segregating for red and white having a larger number in the red-neck class than in the white and cream class*

F_2 bulb No.	F_3 progenies		1:3 ratio		3:13 ratio		F_3 white class	
	Red	White	Deviation	Dev. / P. E.	Deviation	Dev. / P. E.	White and cream	Red neck
46–4	3	7	0.5	.....	1.1	.....	2	5
52–2	12	57	5.2±2.4	2.2	.9±2.2	0.4	18	25
57–4 [a]	71	218	1.3±5.0	.3	16.8±4.5	3.7	72	113
58–2 [a]	14	48	1.5±2.3	.7	2.4±2.1	1.1	19	20
58–3	5	31	4.0±1.8	2.2	1.8±1.6	1.1	9	14
58–4	4	26	3.5±1.6	2.2	1.6±1.4	1.1	4	5
60b–5 [a]	10	29	.3±1.8	.2	2.7±1.6	1.7	6	18
60b–12	35	130	6.3±3.8	1.7	4.1±3.4	1.2	19	26
11c–1	96	334	11.5±6.1	1.9	15.4±5.5	2.8	145	160
Total	250	880	32.5±9.8	3.3	38.1±8.8	4.3	.....	.....

[a] Progeny tests demonstrate that they are 1:3 ratios.

Further evidence in support of the assumption that the segregating F_3 progenies are either 1 : 3 or 3 : 13 ratios of red and white is offered by the F_3 progeny tests of the red classes. All the red individuals in the 1 : 3 ratio should have the genotypic formula *WWii*, while those in the 3 : 13 ratio should have the genotypic formulae *Wwii* and *WWii* in the proportion of 2 : 1. This would yield homozygous red progenies from 1 : 3 ratios and 2 heterozygous red and white progenies to 1 homozygous red progeny from the 3 : 13 ratios. The differentiation also furnishes additional proof that white plants having the genotypic formulae *WWIi* and *WwIi* are present in progenies segregating for red and white in 3 : 13 ratios, since upon self-pollination, white plants with the genetic constitution *WWIi* and *WwIi* should yield the 1 : 3 and 3 : 13 ratios, respectively. The breeding behavior of red individuals from 17 segregating F_3 progenies is presented in Table 8. Of the 17 progenies tested, 6 contained only homozygous red individuals and 11 contained both homozygous and heterozygous red plants. In this case there is a close agreement between the observed and expected ratios, since twice as many *WwIi* genotypes as *WWIi* genotypes are expected in a 3 : 13 ratio.

All of the F_4 progenies which contained less than 12 individuals were not included in the tables because of the uncertainty in classifying the true-breeding red families. The odds against a true-breeding progeny of 12 red individuals being a segregating progeny of 3 red : 1 white are about 22 to 1.

TABLE 8.—*F_4 data from self-pollinated red F_3 onion plants of progenies segregating for red and white in 3 : 13 and 1 : 3 ratios*

F_2 bulb No.	F_3 bulb No.	Progenies from 3 : 13 ratios				F_2 bulb No.	F_3 bulb No.	Progenies from 1 : 3 ratios	
		Segregating F_4 progenies		Nonsegregating F_4 progenies				F_4 progenies	
		Red	White	Red	White			Red	White
	173R-1			30	0		170R-1	45	0
	173R-2			16	0		170R-2	29	0
47-5	173R-3	29	14				170R-3	20	0
	173R-4	11	2				170R-4	56	0
	177R-2			70	0	47-3	170R-5	21	0
	177R-3	39	13				170R-8	24	0
49-2	177R-4	36	17				170R-9	26	0
	177R-5	45	14				170R-10	36	0
	177R-6			56	0		170R-11	33	0
	188R-1			53	0		193R-1a	32	0
	188R-2			40	0		193R-1b	95	0
	188R-3	56	21			52-4	193R-2	15	0
	188R-4	48	16				193R-6	36	0
51-1	188R-6	26	14				200R-1	57	0
	188R-7	11	5				200R-3	55	0
	188R-8			20	0		200R-6	43	0
	188R-9	33	12			53-10	200R-7	67	[a] 1
	188R-10	61	27				200R-8	79	0
	214R-2	21	6				200R-9	42	0
	214R-4	20	5				200R-10	32	0
	214R-5	36	6				234R-1	47	0
	214R-8			14	0		234R-2	131	0
55-5	214R-11a	49	15				234R-23	73	0
	214R-12			39	0		234R-25	13	0
	214R-14	20	6				234R-26	129	0
	214R-15a			18	0		234R-28	122	0
	222R-1	9	3			57-4	234R-29	158	[a] 1
	222R-5			48	0		234R-36	40	0
55-15	222R-6	31	8				234R-37	111	0
	222R-8	22	6				234R-38	92	0
	235R-1	32	16				234R-40	93	0
	235R-2	17	6				234R-41	105	[a] 1
	235R-3	50	15				234R-42	111	0
	235R-4			36	0		237R-1	42	[a] 1
57-5	235R-5	48	18				237R-2	47	0
	235R-6	54	20				237R-3	23	0
	235R-7	11	8				237R-4	27	0
	235R-8	65	22			58-2	237R-5	16	0
	235R-9			21	0		237R-6	13	0
	235R-10			19	0		237R-7	20	0
	236R-1	27	9				237R-8	65	0
	236R-2	20	7				237R-10	18	0
	236R-3			41	0		263R-1	20	0
	236R-4	88	30			60b-5	263R-2	12	0
	236R-6			57	0		263R-3	35	0
58-1	236R-7	46	16				263R-4	37	0
	236R-9	30	10						
	236R-12	43	13						
	236R-13			32	0				
	236R-23	22	5						
	241R-1	77	22						
	241R-2	64	21						
	241R-3	91	28						
	241R-4			70	0				
	241R-6	54	12						
	241R-14	60	17						
59-1	241R-16	30	10						
	241R-19	50	8						
	241R-21	50	19						
	241R-24	43	19						
	241R-25	48	15						
	241R-27			81	0				
	243R-2	31	9						
	243R-3	100	35						
	243R-5	16	7						
	243R-6	55	21						
59-3	243R-7	88	32						
	243R-8	61	22						
	243R-9			67	0				
	243R-10	77	25						
	243R-11	108	35						

TABLE 7.—*F_3 onion progenies segregating for red and white having a larger number in the red-neck class than in the white and cream class*—Continued

F_2 bulb No.	F_3 bulb No.	Progenies from 3 : 13 ratios				F_2 bulb No.	F_3 bulb No.	Progenies from 1 : 3 ratios	
		Segregating F_4 progenies		Nonsegregating F_4 progenies				F_4 progenies	
		Red	White	Red	White			Red	White
9–3	243R–12	53	22						
	243R–14			129	0				
	243R–15			68	0				
59–13	249R–1			16	0				
	249R–2	37	14						
	249R–4	61	18						
	249R–5	20	3						
	249R–6	11	3						
	249R–7	19	8						
	249R–8			31	0				
	249R–9			22	0				
	249R–10	24	16						
	249R–11	53	22						
	249R–12	41	24						
	249R–14	25	13						
60a–16	258R–1	38	13						
	258R–3			17	0				
	258R–4			78	0				
	258R–5	65	19						
	258R–6			74	0				
Total		2,706	937	1,263	0			2,443	0
Theoretical, 3 : 1		2,732.3	910.7						
Deviation		26.3±17.6							
Dev.		1.5							
P. E.									
Total number of F_4 progenies		63		28				46	
Theoretical, 2 : 1		60.7		30.3					
Deviation		2.3±3.0							
Dev.		.7							
P. E.									
Total number of F_2 individuals tested				11				6	
Theoretical, 2 : 1				11.3				5.7	
Deviation				.3±1.3					
Dev.				.2					
P. E.									

[a] Small white sets are not considered, since they might have produced color upon further development.

It is of interest to note the character of the two types of segregating F_3 progenies which have been differentiated by means of the F_3 progeny tests. The data recorded in Table 9 indicate that the F_3 distributions agree with their F_4 behavior. It is true that in the case of the three progenies 53–10, 57–5, and 58–1 the fit of observation to hypothesis is not particularly close, but the progeny tests show that the red individuals include the expected genotypes in the proper proportions.

Self-pollinated red plants from progenies segregating in a 3:13 ratio are recorded in Table 10. Among the 12 red F_2 plants tested 7 were heterozygous and 5 were homozygous for the *Ww* factor pair. This agrees very well with the theoretical expectancy, which is a 2:1 ratio of heterozygotes to homozygotes. These data present additional evidence that the two types of expected reds occur in 3:13 ratios.

TABLE 9.—*F_3 data from selfed white F_2 onion plants of progenies segregating for red and white in 3:13 ratios*

F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		1:3 ratio		F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		1:3 ratio	
		Red	White	Deviation	Dev. / P. E.			Red	White	Deviation	Dev. / P. E.
47–5	*WWii*	4	24	1.3± 1.4	0.9	47–3	*WWii*	14	19	5.7± 1.7	3.3
49–2		10	24	3.6± 1.5	2.4	52–4		11	33	.0± 1.9	0
51–1		79	323	3.6± 5.3	.7	53–10		142	626	50.0± 8.1	6.2
55–5		17	39	6.5± 2.0	3.3	57–4		71	218	1.3± 5.0	.3
55–15		34	155	1.4± 3.6	.4	58–2		14	48	1.5± 2.3	.7
57–5		12	120	12.8± 3.0	4.3	60b–5		10	29	.3± 1.8	.2
58–1		45	352	29.4± 5.3	5.5						
59–1		57	217	5.6± 4.4	1.3						
59–3		22	91	.8± 2.8	.3						
59–13		14	85	4.6± 2.6	1.8						
60a–16		62	302	6.3± 5.0	1.3						
Total		356	1,732	35.5±12.1	2.9	Total		262	973	46.7±10.2	4.6

Progeny No. 50b–1 is considered a homozygous red family on the basis of its progeny tests. Fourteen red individuals were tested and found to breed true for red. Five of the 15 whites were self-pollinated. Four segregated for red and white, giving a total of 16 red and 74 white individuals, and 1 segregated for white and yellow, giving 10 white and 2 yellow bulbs, indicating contamination. Only 3 yellow bulbs have appeared among approximately 10,000 bulbs from crosses Nos. 2 and 3. A mutation from red to yellow is a possible explanation. However, yellow plants were grown near plants Nos. 11–1 and 50b–1, so that crossing may have taken place.

TABLE 10.—*F_3 data from selfed red F_2 onion plants of progenies segregating for 3:13 ratios of red and white*

F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		F_2 bulb No.	F_2 genotypic formulae	F_3 progenies	
		Red	White			Red	White
45–1	*Wwii*	6	2	50b–1	*WWii*	265	[a] 15
45–3		50	20	50–2		12	0
45–4		15	3	50–3		138	0
50–5 [b]		4	0	50–9		23	0
50–10		39	9	11–1		128	[c] 1
50–13 [b]		3	0				
12–7		6	2				
Total		116	36	Total		566	0
Theoretical, 3:1		114	38	Theoretical		566	0
Deviation		2.0±3.6				0	
Dev. / P.E.		.6					

[a] 15 white bulbs not considered (see text).
[b] Progenies not considered because of possible errors in classification.
[c] 1 yellow bulb, which is obviously the result of contamination, since yellow has appeared only twice in progenies derived from crosses No. 2 and No. 3.

TABLE 11.—*F_3 data from selfed red and white F_2 onion plants of progenies segregating for 3:1 ratios of red and white*

F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		Deviation	Dev. / P. E.	F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		Deviation
		Red	White					Red	White	
14c-2	*Wwii*	8	1	1.3		13b-1	*wwii*	0	7	0
14c-3		32	10	.5±1.9	0.3	14-10		0	30	0
62-1		48	10	4.5±2.2	2.0	61-1		0	10	0
						14c-1	*WWii*	18	0	0
						14-1		58	0	0
Total		88	21	6.3±3.1	2.0	Total		76	47	0
Theoretical, 3:1		81.7	27.3							

The data presented in Table 11 demonstrate the presence of the three genotypes *WWii*, *Wwii*, and *wwii*, which would be expected if red and white plants segregating in a 3:1 ratio were self-pollinated. Of the red bulbs tested 3 are heterozygous and 2 are homozygous, which agrees with the expected 2:1 ratio. All the white bulbs behaved like double recessives.

The following crosses were made between tested F_1 bulbs resulting from reciprocal crosses between red and white plants:

Cross No. 20, *WwIi*×*Wwii*.
Cross No. 21, *Wwii*×*WwIi*.
Cross No. 22, *WwIi*×*Wwii*.

Under such circumstances each of the three crosses would form 6 different genotypes in the following proportions:

1 *WWIi*.
1 *WWii*.
2 *WwIi*.
2 *Wwii*.
1 *wwIi*.
1 *wwii*.

The plants which appeared from these crosses are listed in Table 12. Though the numbers from the crosses are necessarily small, the actual ratios agree with the expected ratio of 3 red:5 white.

TABLE 12.—*Data from crosses between F_1 red and white onion bulbs*

Cross No.	Pedigree No.	F_1 progenies		Deviation	Dev. / P. E.
		Red	White		
20	2-2×2-5	8	21	2.9±1.8	1.6
21	2-4×2-1	4	23	6.1±1.7	3.6
22	3-1×3-2	3	3	0.8±0.9	.9
Total		15	47	8.25±2.6	3.2
Theoretical, 3:5		23.25	38.75		

TABLE 13.—*F_2 data from cross No. 20*

F_1 bulb No.	F_1 genotypic formulae	F_2 progenies		Deviation	Dev. / P. E
		Red	White		
69-2	*Wwii*	62	26	4.0±2.7	1.5
69-3	*Wwii*	31	9	1.0±1.9	.5
Total		93	35	3.0±2.7	1.1
Theoretical, 3:1		96	32		
70-1	*wwIi* or *wwii*	0	231	0	0

The F_2 data presented in Table 13 show that expected heterozygous red plants *Wwii* and homozygous white plants *wwii* or *wwIi* were present in the red and white population resulting from cross No. 20.

RED AND YELLOW COLOR RELATIONS

Two different crosses were made between red and yellow open-pollinated stocks of onions. Upon self-pollination the yellow parent that was used in both crosses gave 32 yellow bulbs, demonstrating that it was probably homozygous for factors controlling the production of yellow pigment. Self-pollinations of the two red parents R_{14} and R_{22} were not successful.

TABLE 14.—*F_1 data from crosses between red and yellow onion plants*

Cross No.	Pedigree No.	Parental genotypes	F_1 progenies	
			Red	Yellow
7	$R_{22}\times Y_8$	*WWii*×*WwWii*	6	0
8	$Y_8\times R_{14}$	*WwWii*×*WWii*	6	0
Total			12	0
Theoretical			12	0

In every case the F_1 plants were normal red. (Table 14.) This at once indicated that the gene for red *W* was dominant to the gene or genes for yellow and that the red parents were probably homozygous *WW*.

TABLE 15.—*F_2 and back cross data resulting from crosses between red and yellow onion plants*

F_1 bulb No.	F_1 genotypic formulae	F_2 progenies		Cross No.	Pedigree No.	Parental genotypes	F_1 progenies	
		Red	Yellow				Red	Yellow
7-1	*WWwii*	83	26	23	7-1×6-3	*WWwii*×*WwWwii*	8	9
7-2	*WWwii*	49	23	24	8-2×6-3	*WWwii*×*WwWwii*	8	5
8-1	*WWwii*	69	*16	42	8-1×6-3	*WWwii*×*WwWwii*	8	7
8-2	*WWwii*	32	4					
Total		233	69	Total			24	21
Theoretical, 3:1		226.5	75.5	Theoretical, 1:1			22.5	22.5
Deviation		6.5±5.1		Deviation			1.5±2.3	
Dev.		1.3		Dev.			.7	
P. E.				P. E.				

* One small cream-colored set included.

Only 4 of the 12 red F_1 plants obtained were tested in later generations. These four were all self-pollinated and three were backcrossed to homozygous yellow plants. The progenies of the selfed F_1 plants and back crosses presented in Table 15 show evidence of a simple 3:1 relationship.

The F_1 and F_2 data from crosses Nos. 7 and 8 indicate that a gene for yellow, designated by the symbol W^v, holds a simple Mendelian recessive relationship to the gene W for red.

The F_2 red bulbs should be composed of two kinds of genotypes, WW^vii and $WWii$, and the F_2 yellow bulbs should all have the genetic constitution W^vW^vii. The F_3 progenies presented in Table 16 demonstrate that the three kinds of genotypes were present and that twice as many heterozygous as homozygous red bulbs occurred.

TABLE 16.—*F_3 data from selfed F_2 plants resulting from crosses of red and yellow onion plants*

F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		F_2 bulb No.	F_2 genotypic formulae	F_3 progenies	
		Red	Yellow			Red	Yellow
16c–2	WW^vii	2	3	65–1	W^vW^vii	0	205
17a–1	WW^vii	15	8	65–3	W^vW^vii	0	13
64–2	WW^vii	31	16	65–4	W^vW^vii	0	13
68–2	WW^vii	34	8	67–3	W^vW^vii	0	39
68–3	WW^vii	38	4	[a]14c–1	$WWii$	8	0
				64–1	W^vW^vii	21	0
Total		120	39	Total		29	270
Theoretical, 3:1		119.25	39.75				
Deviation		.8±3.7					
Dev.		.2					
P. E.							

[a] One small white bulb appeared in this progeny which can not be accounted for on the basis of the assumed factorial hypothesis.

Two interesting crosses were made between white F_1 plants that were assigned the genetic formula $WwIi$ in Table 2, and red F_1 plants that were given the genetic formula WW^vii in Table 15. The F_1 families from these crosses, which are listed in Table 17, show three color classes according to the expected 3 red : 1 yellow : 4 white ratio.

The fundamental relations of the factorial interactions are as follows:

$$\begin{matrix} 3 & Wi, \text{ red.} \\ 1 & W^vi, \text{ yellow.} \\ 4 & \begin{cases} WI, \text{ white.} \\ W^vI, \text{ white.} \end{cases} \end{matrix}$$

The progeny tests of the F_1 plants from crosses Nos. 25 and 26 listed in Table 18 show that the four genotypes WW^vii, $WWii$, $Wwii$, and WW^vIi were present. The three progeny tests showing either a 1:3 or 3:13 ratio should account for one or both of the genotypes with the formulae $WWIi$ and $WwIi$. The two expected genotypes, W^vwIi and W^vwii, did not appear among the small number of bulbs tested.

TABLE 17.—*Data from crosses between heterozygous F_1 red and white onion plants*

Cross No.	Pedigree No.	Parental genotypes	F_1 progenies		
			Red	Yellow	White
25	2-1×8-2	*WwIi*×*WWvii*	4	1	6
26	7-2×2-2	*WWvii*×*WwIi*	5	0	6
Total			9	1	12
Theoretical, 3:1:4			8.25	2.75	11.0
Deviation			.75	1.75	1.0

χ^2=1.271. P=0.54.

TABLE 18.—*F_2 data from crosses Nos. 25 and 26*

F_1 bulb No.	F_1 genotypic formulae	F_2 progenies		
		Red	Yellow	White
25a-1	*WWIi* or *WwIi*	13	0	23
25a-3	do	1	0	7
25-5	do	14	0	68
25-3	*WWvii*	46	7	0
Theoretical, 3:1		39.8	13.2	0
Deviation		6.25±2.1		0
71-1	*WWii*	21	0	0
Theoretical		21	0	0
71-2	*Wwii*	242	0	85
Theoretical, 3:1		245.2		81.8
Deviation		3.25±5.3		
72-1	*WWvIi*	3	1	14
Theoretical, 3:1:12		3.4	1.1	13.5
Deviation		.4	.1	.5

χ^2<than 1. P=close fit.

The three crosses between white and yellow listed below were made in an attempt to analyze the white and yellow relationships.

Cross No. 25, 2-1 (*WwIi*)×8-2 (*WWvii*).
Cross No. 26, 7-2 (*WWvii*)×2-2 (*WwIi*).
Cross No. 31, 2-2 (*WwIi*)×6-3 (*WvWvii*).

It is evident that the white plants chosen for this analysis are white because they carry the inhibitor gene *I*. The tests resulting from these crosses show that the *Ii* factor pair holds the same relation to the gene for yellow, *Wv*, as it does for the gene for red, *W*. However, these crosses are not designed to give information as to the relationship between the gene for yellow, *Wv*, and the gene for white, *w*, which is necessary in order to demonstrate that the geres *W*, *Wv*, and *w* form an allelomorphic series.

TABLE 19.—*Data from a cross between a heterozygous F_1 white onion plant and a homozygous yellow onion plant*

Cross No.	Pedigree No.	Parental genotypes	F_1 progenies		
			Red	Yellow	White
31	2-2×6-3	*WwIi*×*WvWvii*	6	[a]1	6
Theoretical, 1:1:2			3.25	3.25	6.5
Deviation			2.75	2.25	.5

χ^2=3.95. P=0.14.
[a] A questionable yellow bulb.

The F_1 data presented in Table 19 involve a cross between the tested white F_1 plant No. 2–2 (see Tables 2 and 10) that was given the genotypic formula *WwIi*, and a true-breeding yellow plant No. 6–3 that may be assigned the genotypic formula W^vW^vii, since on selfing it yielded 47 yellow bulbs. The poor fit observed in this case might have been influenced by errors in classification of the 13 bulbs, 5 were too small to insure accurate classification at harvest time. For this reason 7 of the 13 bulbs were grown in the greenhouse during the following winter months for the purpose of increasing their size so that an accurate classification could be made and seed production assured for the following year. While the bulbs were growing a reclassification was made in which one bulb was classified as a questionable yellow. It might be of interest to note that at the time a factorial hypothesis had not been formulated, which demanded a yellow class in this population. Only 3 of the 13 plants lived and produced seed the following year.

In self-pollinated progenies from the white F_1 plants the ratios should be either 3 yellow : 13 white or 3 red : 1 yellow : 12 white. The ratios in three F_2 progenies recorded in Table 20 are obviously 3 red : 1 yellow : 12 white, resulting from F_1 plants with the genotypic formula WW^vIi.

TABLE 20.—*F_2 data from cross No. 31*

F_1 bulb No.	F_1 genotypic formulae	F_2 progenies		
		Red	Yellow	White
31a–1	WW^vIi	20	2	87
31–6	WW^vIi	8	10	39
31b–1	WW^vIi	26	11	103
Total		54	23	229
Theoretical, 3:1:12		57.4	19.1	229.5
Deviation		3.4	3.9	.5

$\chi^2 = 0.998$. P = close fit.

A fairly large number of F_3 progeny tests were made of the F_2 individuals resulting from the selfed white F_1 bulb 31–6. These F_2 plants should include 9 different genotypes in the following proportions:

1 *WWII*.
2 WW^vII.
2 *WWIi*.
4 WW^vIi.
1 W^vW^vII.
2 W^vW^vIi.
1 *WWii*.
2 WW^vii.
1 W^vW^vii.

The presence of six of these genotypes is definitely demonstrated by the F_3 progeny results presented in Table 21. The three true-breeding white genotypes *WWII*, WW^vII, and W^vW^vII, may be represented, although it is not possible to differentiate between them.

TABLE 21.—*F_3 data from cross No. 31*

F_2 bulb No.	F_2 genotypic formulae	F_3 progenies		
		Red	Yellow	White
404W–4	*WWvIi*	22	6	83
404W–6	do	28	9	113
404W–10	do	25	6	79
Total		75	21	275
Theoretical, 3:1:12		69.6	23.2	278.2
Deviation		5.4	2.2	3.2
404R–1	*WWvii*	72	22	[a]1
404R–2	do	53	18	0
Total		125	40	0
Theoretical, 3:1		123.75	41.25	0
Deviation		1.25±3.8		
404R–3	*WWii*	49	0	0
Theoretical		49	0	0
404Y–1	*WvWvii*	0	58	0
Theoretical		0	58	0
404W–1	*WWII* or *WWvII* or	0	0	200
404W–2	*WWII* or *WvWvII*	0	0	28
404W–11	do do	0	0	338
Total		0	0	566
Theoretical		0	0	566
404W–3	*WWIi*	93	0	271
404W–7	do	115	0	402
404W–8	do	38	0	101
404W–9 [b]	*WWIi* or *WWvIi*	1	0	12
Total		246	0	774
Theoretical, 1:3		255	0	765
Deviation		9±9.4		
404W–5	*WvWvIi*	0	45	99
Theoretical, 1:3		0	36	108
Deviation			9.0±3.5	

χ^2=0.665. P=close fit.
[a] One white set not included since it may have developed yellow color if allowed to mature.
[b] This progeny was not considered because it might be the result of either *WWvIi* or *WWIi* genotype.

When a heterozygous red F_1 plant from the original white and red cross, $W_{22} \times R_{11}$, is crossed with another heterozygous red F_1 plant from a red and yellow cross the resulting population should be composed of the following genotypes in equal proportions:

1 *WWii*
1 *WWvii* } 3 red.
1 *Wwii*
1 *WvWvii* 1 yellow.

The red and yellow proportions resulting from crosses Nos. 27 and 28 agree with the factorial analyses mentioned above. Progeny tests from the two crosses listed in Table 22 could not be made owing to storage-rot losses.

TABLE 22.—*Data from crosses between heterozygous red F_1 onion plants*

Cross No.	Pedigree No.	Parental genotypes	F_1 progenies	
			Red	Yellow
27	3-2×8-2	*Wwii*×*WWvii*	10	4
28	8-1×2-4	*WWvii*×*Wwii*	13	2
Total			23	6
Theoretical, 3:1			21.75	7.25
Deviation			1.25±1.6	

RELATION OF COLOR FACTORS TO DISEASE RESISTANCE

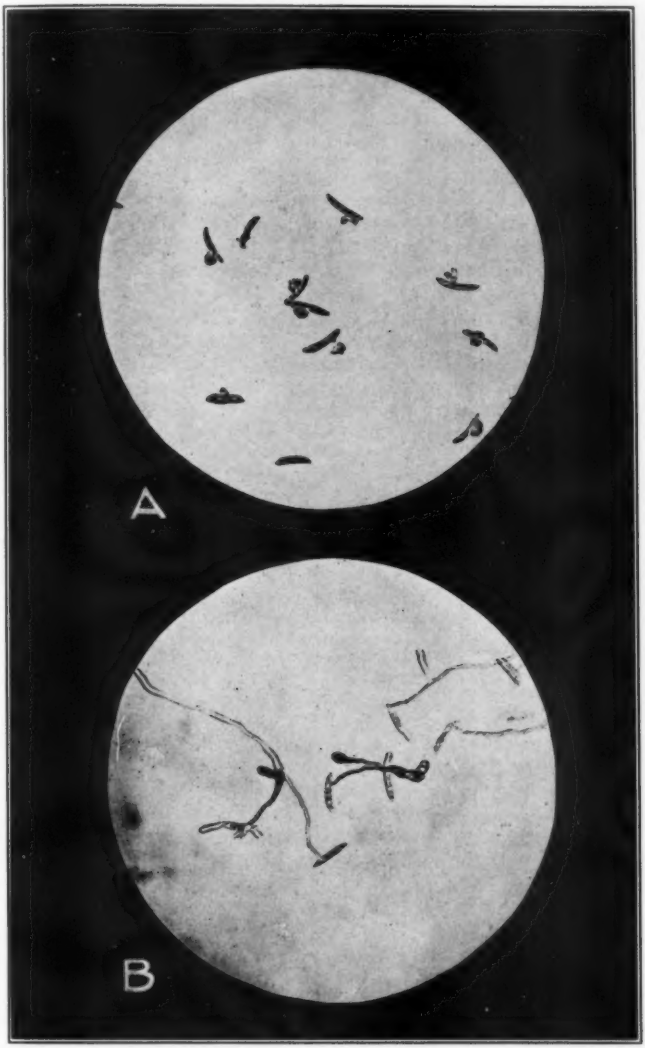
The pedigreed stocks of onion bulbs discussed in this paper were subjected to field and laboratory tests to determine their resistance to the onion-smudge disease. As stated above, the field tests were not entirely satisfactory for the classification of bulbs into grades of resistance because of variation in the amount of inoculum and the influence of soil and environmental conditions upon infection. In general, however, the red-neck bulbs tended to show an intermediate stage between the high resistance of the red and the extreme susceptibility of pure white bulbs. The laboratory tests were used to determine the relative amounts of toxic material in the scales, which has been shown to be responsible for the resistance of red bulbs (*5, 10, 12*). The methods employed have already been described (p. 254).

When bits of dry red or yellow scales from either homozygous or heterozygous bulbs were placed in drops of spore suspension of *Colletotrichum circinans*, distinctly abnormal germination occurred. (See Pl. 3.) These abnormalities may be divided into three general classes: (1) Rupturing of the germ tube, characterized by a short ruptured germ tube from which a portion of the spore contents exudes in the form of a naked mass; (2) swelling of the spore; (3) thickening of the germ tube, characterized by a blunt thick-walled tube without an appressorium. Many spores failed to show changes in form, which might indicate that they were resistant to the toxic substances found in red scales. Drops of spore suspensions that had been treated with red scale tissue for 24 to 48 hours were shaken from the glass slide and several drops of a 1 per cent solution of dextrose were added to the smear. None of the normally shaped spores showed evidence of germination, whereas the controls, containing an additional lot of fresh spores, exhibited normal germination, showing that those which did not germinate had been killed by the toxic substances.

TABLE 23.—*Data showing toxicity of dry outer scales of F_3 onion bulbs from reciprocal crosses between red and white as related to smudge resistance*

F_3 progeny No.	F_3 phenotype	F_3 ratio	Bulbs tested	Resistant	Intermediate resistant	Susceptible
234	Red	71 red : 218 white	18	18	0	0
234	Red neck	do	137	0	29	108
234	White and cream	do	50	0	4	46
Total			205	18	33	154
178	White and cream	47 white	25	0	0	25
191		374 white	25	0	1	24
202		326 white	25	0	0	25
206		14 white	12	0	0	12
221		604 white	7	0	0	7
224		132 white	25	0	0	25
227		30 white	25	0	0	25
231		105 white	32	0	2	30
246		90 white	25	0	1	24
255		40 white	25	0	2	23
259		83 white	25	0	1	24
Total			251	0	7	244

Red and white F_3 bulbs from crosses Nos. 2 and 3 were tested for the presence of toxic materials in the outer dry scales. For convenience the tested bulbs presented in Tables 23 and 24 are grouped



EFFECT OF COLD WATER EXTRACT FROM DRY OUTER SCALES OF COLORED ONIONS UPON SPORE GERMINATION OF COLLETOTRICHUM CIRCINANS

A, Photomicrograph of spores about 17 hours after being placed in an extract from yellow scales. Note the masses of naked cytoplasm which have exuded from the spores after the tips of the germ tubes have ruptured. Compare with B; B, photomicrograph of spores about 17 hours after being placed in an extract from white scales. Note the normal germ tubes and appressoria. Compare with A.

empirically in the following classes: Resistant, intermediate resistant, and susceptible, according to the amount of toxic materials indicated by this spore-germination test. The individuals classified as intermediate resistant were tested twice to make certain that they contained materials which were toxic to the smudge organism. The bulbs that caused somewhat abnormal germ-tube development but allowed a high percentage of the spores to germinate and grow are included in the susceptible class.

In Table 23, the analysis of a progeny segregating for red and white shows that the proportion of intermediate resistant to susceptible bulbs in the red-neck class is approximately 1:4, whereas in the white-and-cream class it is about 1:12. In progenies breeding true for white, the proportion of intermediate resistant to susceptible is about 1:35. The data recorded in Table 24 also show a high correlation between the heterozygous red-neck character and intermediate toxicity. Approximately one bulb in three is partially resistant in the red-neck class, whereas in the white class the bulbs are all extremely susceptible. The three intermediate resistant bulbs occurring in a population of 20 cream bulbs are very deep cream in color, and may be the result of an interaction between the yellow gene W^y and the inhibitor gene *I*.

TABLE 24.—*Data showing toxicity of white F_2 bulbs from cross No. 31 between white and yellow onion plants as related to smudge resistance*

F_2 progeny No.	F_2 phenotype	F_2 ratio	Bulbs tested	Intermediate resistant	Susceptible
301	Red neck	20 red : 2 yellow : 87 white	30	7	23
301	Cream	do	20	3	17
301	White	do	20	0	20

The data presented in Tables 23 and 24 indicate that white intermediate resistant bulbs occur more frequently in progenies segregating for red and white than in progenies breeding true for white. This relationship would be expected if the heterozygous factor pair *Ii* shows incomplete epistatis over the *W* and W^y genes (responsible for production of at least one substance toxic to the smudge organism), and the homozygous factor pair *II* usually shows complete epistatis. In other words, the appearance of a partially resistant class among white onion bulbs is undoubtedly due in a large measure to the presence in those bulbs of one of the resistant genes, *W* or W^y, but the expression of resistance by these genes is partially inhibited by the second factor pair *Ii*. The variability in the expression of the red-neck character should reflect a corresponding variation in the production of toxic materials. This relationship and the fact that the toxic substances are water soluble and subject to leaching explains, in part, how genetically white bulbs possessing heterozygous *Ii* factor pairs may vary in their reaction to disease. There is also the possibility of other modifying factors acting on the expression of disease resistance and on the expression of the heterozygous character, red neck.

DISCUSSION AND CONCLUSIONS

Perhaps the most interesting contribution in this paper is the proof of the existence of two genes for resistance to smudge in onions, each having the ability to produce a definite water-soluble chemical entity, protocatechuic acid, which is extremely toxic to the causal organism, *Colletotrichum circinans*. Besides the production of a colorless water-soluble toxin, these two genes, *W* and W^v, are responsible for the production of red and yellow pigments, respectively, which contain the water-insoluble pigment, quercetin. It has been demonstrated (*5*) that upon alkaline fusion quercetin yields two toxic chemicals, oxalic acid and protocatechuic acid, and one nontoxic chemical, phloroglucinol. Oxalic acid probably does not contribute directly to the resistance of pigmented bulbs, for numerous attempts to isolate free oxalic acid from colored scales have failed.

Since the inheritance of disease resistance in this case involves the inheritance of certain pigments, any knowledge of the nature of disease resistance must of necessity be concerned with the relations between the chemistry and biochemistry of the plant pigments and the genetic factors for color inheritance.

The early investigations on the chemistry of plant pigments were carried out independently of the Mendelian point of view. Likewise, the first botanists and geneticists who turned their attention to the inheritance of color in plants failed to emphasize the fact that studies on the inheritance of color in reality involved studies on the inheritance of chemical compounds and chemical reactions. Wheldale (*13*, *14*, *15*, *16*) was the first to point out this relationship and later Keeble and Armstrong (*2*, *3*) and Keeble, Armstrong and Jones (*4*) added further details. The work published by Wheldale and Bassett (*17*, *18*) on the pigment of the snapdragon (*Antirrhinum majus*) has shown yellow to be identical with the known substance luteolin, and ivory with the known substance apigenin. The pigment in rose doré was shown to be a red anthocyanin and the same pigment mixed with yellow gives the bronze color; similarly magenta contains a magenta anthocyanin, and this mixed with yellow gives crimson.

All the varieties may be expressed in terms of the following factors:

Y—A factor leading to the production of luteolin in the lips and apigenin in the tube.
I—A factor leading to the suppression of luteolin in the lips, apigenin being formed instead.
L—A factor leading to the production of a tinge of red pigment in the lips.
T—A factor leading to the production of a tinge of red pigment in the tube.
D—A factor leading to the production of more anthocyanin pigment, i. e., a deepening or full-color factor.
B—A factor converting red into magenta anthocyanin.

Thus the genes involved in flower colors in Antirrhinum can be expressed by: (1) The synthesis of definite chemical substances; (2) the modification of such substances once they are formed; (3) the control of enzyme action; (4) the modification of cell-sap reaction.

Onslowe's (*7*) chemical interpretation of the interaction of the *Y* gene and the *I* gene in Antirrhinum may prove helpful in an elucidation of the chemistry of closely related pigments in the common onion that are governed by similar factorial interactions. The formula *YYII* has been assigned to the ivory-colored flower, *YYii* to the yellow-colored flower, and *yyii* (or *II*) to the albino flower.

The *I* gene may be considered as responsible for the production of a substance that inhibits the formation of luteolin, or, since luteolin is more highly oxidized than apigenin, *I* may be a reducing agent. On treatment with caustic alkali apigenin forms *P*-oxybenzoic acid, whereas luteolin yields protocatechuic acid. Thus, an inhibitor gene may operate in Antirrhinum to control the formation of the organic acid, which, when present in the onion, contributes toward resistance to the smudge disease.

Neither the chemical nor the genetical work on onion pigments has shown how the toxic materials associated with color are formed. It is of course helpful to know that at least one toxic substance found in resistant bulbs can be produced as a decomposition product by alkaline fusion of the flavonol quercetin.

The question as to the mode of formation of the toxic materials in the outer scales of onion bulbs is of primary importance from the standpoint of breeding for disease resistance. From the evidence at hand it can be said that although the pigmented condition in the onion is closely correlated with resistance, the toxic substance which has been isolated is colorless. This suggests the possibility that toxins may be formed without a corresponding development of red and yellow pigments. The interaction of the incomplete inhibitor of color, gene *I*, with the genes for color, *W* and W^v, lends support to this assumption. White bulbs with the genetic constitution *WWIi* and *WwIi* show an intermediate toxicity to the disease. The bulbs possessing the heterozygous *Ii* factor pair and genes *W* or W^v for color are neither red nor yellow but usually show light cream-colored outer scales. Occasional intermediate resistant white bulbs were observed in true-breeding white progenies, which indicates a possibility of obtaining resistant varieties of white onions.

In the light of our present knowledge the possibility of obtaining white resistant bulbs may be discussed from at least two different points of view:

1. If protocatechuic acid or other toxic substances are formed in resistant bulbs as precursors of the pigment quercetin then it is reasonable to expect that the *I* inhibitor gene in the presence of resistant color genes may permit the formation of toxins and suppress the formation of pigments. If, however, the toxins are decomposition products of the pigment quercetin, then it becomes difficult to conceive how white bulbs may possess resistance.

2. The *I* gene may interact with the color genes in such a manner as to produce a condition resulting in the production of a colorless isomer in the place of the yellow pigment quercetin. This would permit resistance in white bulbs even though the toxins were decomposition products. Willstätter (*19*) reports that many of the anthocyanins that are related to flavonols can readily be induced to change to colorless isomers. Whatever the nature of the systems producing toxins may be, it is more than likely that the complexes of chemical substances which produce the different expressions of disease resistance are the result of the activity of different "patterns of protoplasm," governed, in part at least, by the chromosomes.

Genetic analyses of disease resistance have been highly successful in those cases where the factorial interactions are manifested by the appearance of only two classes—resistant and susceptible. A factorial analysis of those cases where the reaction of the host to the parasite is evidenced by a range of types intermediate between

complete susceptibility and complete resistance is exceedingly difficult because of the necessity of employing arbitrary genetic classes. In this investigation the genetic formulae of the intermediate resistant plants are determined by the breeding behavior of the red, yellow, cream, and white characters. If the factors for disease resistance in the onion were not associated with color characters then it would not be possible to formulate an accurate genetic analysis of the intermediate resistant classes. A classification based solely on resistance tests would not indicate clearly the presence of an inhibitor of resistance gene. It is therefore of interest to note that, by the aid of color characters, it has been possible in this study to demonstrate one of the first cases of a satisfactory factorial analysis of intermediate resistance.

The data presented in this report have demonstrated that the various colored onions possess the following factors:

Red	*Wi.*
Yellow	$W^{y}i$.
White	*wi* and *II.*
Red neck	*WIi.*

In progenies resulting from crosses between dominant white and red the presence of six of the nine different genotypes expected in a 13:3 ratio have been demonstrated, namely,

WwIi.
$WW^{y}Ii$.
Wwii.
$ww^{y}Ii$.
wwii.
WWii.

In F_3 progenies resulting from crosses between a white plant with the genetic formula *WwIi* and a yellow plant with the genetic formula $W^{y}W^{y}ii$ the presence of six of the nine different genotypes expected in a 12:3:1 ratio have been demonstrated, namely,

WWIi.
$WW^{y}Ii$.
WWii.
$WW^{y}ii$.
$W^{y}W^{y}Ii$.
$W^{y}W^{y}ii$.

All of the genetic relations presented in this article strongly indicate that the genes *W*, W^{y}, and *w* are members of an allelomorphic series. However, only two of the three critical tests necessary to demonstrate an allelomorphic relationship of three genes were made. In the absence of the inhibitor gene *I*, the gene *W* for red and the gene *w* for white show a simple monohybrid condition. Likewise gene *W* for red and gene W^{y} for yellow show a simple monohybrid relationship. When the attempt was made to test out the yellow and white relationship the color-inhibiting gene *I* was not recognized, and white plants carrying this factor were used in the crosses. The final proof of the existence of a multiple allelomorphic color series in the onion depends, therefore, upon demonstrating the allelomorphism between W^{y} and *w* genes.

SUMMARY

In an attempt to make a genetic analysis of the relation of the red, yellow, and white color classes to disease resistance in the common onion, data involving a classification of not less than 330 prog-

enies and approximately 22,000 individual bulbs are reported. An interpretation of the results obtained in the F_1 to the F_4 generations of the various genetic tests employed requires five different genes governing bulb pigmentation as follows:

(1) *I* gene for incomplete inhibition of color.
(2) *i* gene allowing expression of color.
(3) *W* gene for red pigment.
(4) W^y gene for yellow pigment.
(5) *w* gene for white.

The inhibitor of color, gene *I*, is dominant to its recessive allelomorph *i*. The heterozygous factor pair *Ii* produces red-neck and cream-colored bulbs in the presence of the color genes *W* or W^y. Independent inheritance is demonstrated between the allelomorphic pair *Ii* and the genes for color.

The gene for red, *W*, is dominant to the gene for yellow, W^y, and the gene for white, *w*. It is assumed that the gene *W* is allelomorphic to the genes W^y and *w*. The relation between genes W^y and *w* has not been demonstrated.

The genes *W* and W^y are responsible for the production of a toxic substance, protocatechuic acid, in the outer scales of resistant onions.

The color-inhibitor gene, *I*, interacts with the gene for red, *W*, producing intermediate resistant bulbs.

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EXTENSION OF PEARSON'S CORRELATION METHOD TO INTRACLASS AND INTERCLASS RELATIONSHIPS [1]

By J. ARTHUR HARRIS,[2] *Head, Department of Botany, University of Minnesotat and Collaborator, Office of Western Irrigation Agriculture, Bureau of Plan, Industry, United States Department of Agriculture,* and BORGHILD GUNSTAD, *Assistant in Biometry, Department of Botany, University of Minnesota*

INTRODUCTION

Because of the inadequacy of available data, the biometrician sometimes has to determine in terms of the universally comparable scale of the correlation coefficient the relationship between two variables, one of which is classed in two alternative categories only. In such cases information concerning one variable, A, is recorded in multiple classes in terms of a quantitative scale or in multiple nonquantitative categories, while information for the other variable, B, is limited to the percentage of cases wherein B exceeds or falls short of a given intensity for each grade or class of A.

For the purposes of the present discussion such biserial tables are most conveniently thought of as originating from the condensation in one direction of the ordinary correlation or contingency surface.

A correlation surface having both B and A expressed in several quantitatively measured classes may be condensed by combining all the values of B into two alternative groups for each of the several classes of A. The result will be the biserial correlation table. Pearson (*13*) [3] showed how this relationship (biserial r) may be expressed in terms of the correlation scale.

In like manner a contingency surface having both B and A expressed in several nonquantitative categories may be condensed by combining all the values of B into two alternative groups for each of the several categories of A. The result will be the double-row contingency table. Pearson (*14*) has given methods for dealing with this relationship (biserial η) also.

When the biserial correlation or contingency table is still further reduced by combining A as well as B in two alternative categories, the limit of condensation is reached in the (2×2)-fold table. Correlation and contingency coefficients have been derived from such tables in various ways. Pearson has expressed the relationship in terms of the equivalent-probability correlation coefficient (*15*).

Such extreme condensation [4] of the correlation or contingency surface as that which results when correlation or contingency tables with a reasonably large number of classes of both A and B are reduced to the biserial correlation or contingency distribution (and especially when such biserial distributions are further condensed from a $(2\times n)$-fold to a (2×2)-fold distribution) must of necessity limit our knowl-

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[3] Reference is made by number (italic) to Literature Cited, p. 290.

[4] In the case of the contingency surface, and in the case of the correlation surface when treated by the correlation-ratio method, the grouping may be made too fine as well as too coarse to give trustworthy values of the coefficient. It is for this reason that "extreme" condensation is specified.

edge of the interrelationships between the two variables. The process is not indicated with any purpose of recommending it in cases in which the extended correlation or contingency surfaces are available, but merely to make clear the nature and the limitations of the biserial distributions as they must be considered in the present discussion.

The applicability of the correlation and contingency methods is not limited to the relationship between the magnitudes of A and B as measured on the same individual or on two individuals that are associated for some logical reason. In some cases, groups of individuals are associated in classes or in pairs of homologous classes, and it may be desirable to determine the direct intraclass correlation coefficient $r_{A_1A_2}$ and $r_{B_1B_2}$, the cross intraclass correlation coefficient $r_{A_1B_2}$, or the direct, $r_{A_1'A_2'}$ and $r_{B_1'B_2'}$, or cross $r_{A_1'B_2'}$, interclass correlation or contingency coefficients. Here the numerical subscripts denote a first and a second individual of the same class or of different but homologous classes, in the sense that each individual is used once as a first and once as a second member of a pair in the weighted correlation or contingency surface. The subscripts A and B without primes indicate individuals of the same class; those with primes denote individuals of different classes.

Such intraclass or interclass correlation or contingency tables may be readily formed by a procedure indicated elsewhere (*2*), or condensed[5] correlation tables (*3*) may be formed; finally, by employing the method of moments about 0 as origin (*1*), the intraclass and interclass coefficients may be calculated directly from the moments and product moments of the classes (*4*).

In a recent paper (*10*) attention has been called to the advantage of employing intraclass equivalent-probability correlation coefficients for certain cases in which the characteristics of the individual may be expressed in alternative categories only. Methods of determining the (2×2)-fold tables for such coefficients from class moments have been given recently (*8*).

The purpose of the present paper is to consider methods whereby biserial intraclass and interclass coefficients may be determined directly from the moments of the classes and subclasses about 0 as origin.

THEORETICAL DEVELOPMENT OF METHOD, WITH PRACTICAL APPLICATION

Let a given lot of N individuals be divided for some logical reason into M classes. Let there be m subclasses in each class and n individuals per subclass. Further, let the n_s and n_d individuals comprising any subclass fall into the alternative categories, s and d,[6] respectively.

The individuals of the M classes may be all that are available, in which case only the intraclass correlation can be determined. In other cases there may be another system (or other systems) of

[5] "Condensed" is here used in a sense somewhat different from that employed in reference to the biserial tables in the preceding paragraphs.

[6] Since biserial intraclass and interclass coefficients will probably be most frequently applied in cases in which the division is into the two classes "survived" and "died," s and d may be used as symbols to denote the fate of an individual.

M homologous classes, each divided into μ subclasses of $\nu_s + \nu_d = \nu$ individuals (of which ν_s have the character s and ν_d have the character d) and comprising a total of $\Sigma(\nu)$ individuals per class. In such a case the intraclass correlation for the variates of the second system of classes and the interclass correlation for the variates of the first and second systems can also be determined.

For any one of the n (or ν) individuals of any subclass only two alternative possibilities, s and d, may be realized. The other $(m-1)$ (or $\mu-1$) subclasses of the same class, or the μ subclasses of the homologous classes of another system, are measured on a quantitative scale in terms of n_{s_p}/n_p, n_{s_q}/n_q, n_{s_r}/n_r etc., or in terms of ν_{s_p}/ν_p, ν_{s_q}/ν_q, ν_{s_r}/ν_r, etc., for the pth, qth, rth, etc., subclass, or, if n or ν be constant for all the m or μ subclasses of the class, in the quantitative terms of n_{s_p}, n_{s_q}, n_{s_r}, etc., ν_{s_p}, ν_{s_q}, ν_{s_r}, etc., for these respective classes.

The problem now presented is twofold: (1) To determine the correlation between the fate of any individual in any subclass of a class, as expressed in alternative categories, and the characteristics of the $(m-1)$ subclasses of the same class, expressed in quantitative terms of n_s/n or, if n be constant, of n_s (or in comparable values of ν). The result is the biserial intraclass correlation coefficient. (2) To determine the correlation between the fate of any individual of the n in a subclass of one system, as expressed in alternative categories, and the characteristics of the individuals of an homologous class of another system of classes, as measured in the quantitative terms of ν_s/ν, or, if ν be constant, of ν_s. The result is the biserial interclass correlation coefficient.

Since there are n individuals in a subclass, the arrays of values of n_s[7] associated with the alternative categories s and d into which the individuals of any subclass may be thrown must be given a total weighting of n. Individuals of character s will have associated with them the array of $(m-1)$ subclasses weighted in an n_s-fold manner; individuals of character d will have associated with them the array of $(m-1)$ subclasses weighted in an $(n-n_s)$-fold manner, or in an n_d-fold manner.

The problem may be clarified by an illustration. Table 1 gives the number of seedlings per hill (subclass) produced on 48 associated pairs of subplots, of 5 hills each, planted to sea-island and Durango cotton. This table represents an actual mapping of the data of experiment 2/25, plot D–2–4, of the United States Field Station, Sacaton, Ariz., summarized by Harris, Harrison, and Wadley (*9*, *Table 8*) and further discussed by Harris and Ness (*10*).

The symmetrical intraclass correlation surface for the sea-island variety is given as Table 2. The interclass correlation surface, measuring the relationship between the number of seedlings per subclass in each class of the sea-island variety and per subclass in each of the homologous classes or plots of the Durango variety, is given as Table 3.

[7] Quite obviously the arrays may be in terms of n_d/n instead of in terms of n_s/n, or in terms of n_d instead of n_s, if it is for any reason more desirable.

TABLE 1.—*Number of seedlings per subclass (hill) on 48 adjoining pairs of subplots of sea-island and Durango upland cotton, together with the moments necessary for calculating biserial intraclass and interclass correlation coefficients based on data from cultures at the United States Field Station, Sacaton, Ariz., 1925 (experiment 2/25, plot D–2–4)*

Subplot No.	Sea-island cotton on—								Durango cotton on—							
	Hill (subclass) No.					Σn_s	Σn_s^2	Σn_s^3	Hill (subclass) No.					Σp_s	Σp_s^2	Σp_s^3
	1	2	3	4	5				1	2	3	4	5			
4–1	4	1	6	5	5	21	103	531	5	6	5	4	4	24	118	594
4–2	0	5	4	3	4	16	66	280	1	3	2	5	5	16	64	286
4–3	0	0	0	0	0	0	0	0	1	0	0	4	5	10	42	190
4–4	1	0	0	0	0	1	1	1	3	1	4	0	0	8	26	92
4–5	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
4–6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4–7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4–8	5	6	0	0	0	11	61	341	0	0	4	3	4	11	41	155
8–1	5	6	6	6	6	29	169	989	6	5	5	4	6	26	138	746
8–2	4	3	6	5	4	22	102	496	4	3	6	4	2	19	81	379
8–3	5	5	3	5	4	22	100	466	4	5	5	6	5	25	127	655
8–4	6	3	5	6	6	26	142	800	6	3	5	6	4	24	122	648
8–5	4	3	1	0	5	13	51	217	4	5	5	3	4	21	91	405
8–6	5	6	6	2	5	24	126	690	3	6	5	4	6	24	122	648
8–7	1	5	5	5	6	22	112	592	5	4	1	2	6	18	82	414
8–8	3	2	5	1	1	12	40	162	4	4	3	5	6	22	102	496
12–1	5	5	4	1	4	19	83	379	0	0	6	3	3	12	54	270
12–2	3	5	3	2	0	13	47	187	0	0	0	3	0	3	9	27
12–3	1	5	4	2	6	18	82	414	5	0	0	0	4	9	41	189
12–4	3	4	1	3	4	15	51	183	1	4	5	3	3	16	60	244
12–5	0	0	0	0	3	3	9	27	1	3	6	3	1	14	56	272
12–6	0	0	0	0	5	5	25	125	0	0	0	1	3	4	10	28
12–7	5	6	6	6	3	26	142	800	5	5	4	0	3	17	75	341
12–8	5	4	6	5	6	26	138	746	5	5	5	4	3	22	100	466
16–1	1	3	5	2	1	12	40	162	4	2	2	1	0	9	25	81
16–2	3	4	5	5	5	22	100	466	4	4	0	4	3	15	57	219
16–3	5	4	6	6	5	26	138	746	5	3	6	3	4	21	95	459
16–4	5	6	6	2	0	19	101	565	4	5	4	4	5	22	98	442
16–5	5	6	3	4	5	23	111	557	4	4	5	6	5	24	118	594
16–6	4	3	5	6	2	20	90	440	1	5	5	4	3	18	76	342
16–7	4	5	6	2	3	20	90	440	5	6	5	3	6	25	131	709
16–8	3	6	6	4	6	25	133	739	5	5	5	4	5	24	116	564
20–1	4	5	2	6	5	22	106	538	5	5	0	3	5	18	84	402
20–2	6	5	3	2	4	20	90	440	2	5	4	6	5	22	106	538
20–3	0	3	6	5	6	20	106	584	2	3	6	6	6	23	121	683
20–4	5	4	6	2	5	22	106	538	6	1	4	4	5	20	94	476
20–5	6	3	4	4	5	22	102	496	5	2	5	5	6	23	115	599
20–6	4	2	5	4	6	21	97	477	5	4	2	5	1	17	71	323
20–7	5	5	5	5	4	24	116	564	2	0	6	4	5	17	81	413
20–8	4	5	5	4	0	18	82	378	6	2	1	4	2	15	61	297
24–1	2	4	4	2	4	16	56	208	3	4	2	0	0	9	29	99
24–2	0	3	0	0	0	3	9	27	2	3	1	0	0	6	14	36
24–3	0	0	0	4	1	5	17	65	5	4	5	0	0	14	66	314
24–4	2	4	1	0	1	8	22	74	4	1	0	0	0	5	17	65
24–5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24–6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24–7	0	0	0	3	0	3	9	27	3	0	1	0	0	4	10	28
24–8	0	0	0	0	0	0	0	0	0	2	0	0	0	2	4	8
Total						715	3,371	16,957						699	3,151	15,231

TABLE 2.—*Intraclass correlation table for number of seedlings per subclass (hill) in sea-island cotton (experiment 2/25, plot D–2–4)*

SEA-ISLAND		SEA-ISLAND							
		0	1	2	3	4	5	6	Total
SEA-ISLAND	6	7	3	11	17	21	45	40	144
	5	14	13	15	22	43	48	45	200
	4	9	10	15	18	20	43	21	136
	3	17	7	7	4	18	22	17	92
	2	3	7	2	7	15	15	11	60
	1	10	6	7	7	10	13	3	56
	0	212	10	3	17	9	14	7	272
	Total	272	56	60	92	136	200	144	960

TABLE 3.—*Interclass correlation table for number of seedlings per subclass (hill) in associated classes of sea-island and Durango cotton (experiment 2/25, plot D–2–4)*

SEA-ISLAND

DURANGO	0	1	2	3	4	5	6	Total
6	8	5	7	14	17	39	35	125
5	17	11	17	28	47	66	64	250
4	25	17	16	20	34	58	40	210
3	31	8	8	20	23	29	21	140
2	13	5	6	8	17	19	7	75
1	37	7	5	9	11	12	4	85
0	209	17	16	16	21	27	9	315
Total	340	70	75	115	170	250	180	1,200

Such a symmetrical intraclass correlation surface as Table 2 may be condensed into a biserial distribution of arrays associated with the alternative categories into which any one individual of any subclass may be thrown. This may be done by combining either the columns or the rows (arrays) of frequencies which constitute the surface, after weighting each frequency of the array in an n_s-fold manner to form the arrays of n_s associated with s and in an $(n\text{-}n_s)$-fold manner (or in an n_d-fold manner) to form the arrays of n_s associated with d in the biserial distribution.

The results are given in Table 4. While it shows no superficial evidence of symmetry, this biserial distribution is fundamentally symmetrical in that each variate is used once as a first and once as a second variable. It is fundamentally asymmetrical in that the first variable is weighted and expressed in the alternate categories in which the individual seeds fall, whereas the second variable is expressed on the quantitative scale of number of seedlings per subplot.

TABLE 4.—*Biserial intraclass correlation table for number of seedlings per subclass (hill) in sea-island cotton (experiment 2/25, plot D–2–4)*

SEA-ISLAND

SEA-ISLAND	0	1	2	3	4	5	6	Total
S	215	164	233	317	515	791	625	2,860
D	1,417	172	127	235	301	409	239	2,900
Total	1,632	336	360	552	816	1,200	864	5,760

The interclass correlation surface showing the relationship between the number of seedlings of sea-island and Durango cotton produced in the two associated (homologous) classes (Table 3) is not, and should not be made, a symmetrical table, since the two variables may be differentiated.

Since the two varieties may be differentiated, *two* biserial tables may be derived from this surface, one in which seeds of sea-island cotton are considered to fall in the two alternative categories, s and d, and the other in which the seeds of Durango cotton are classified in comparable alternative categories. Thus while the intraclass correlation surface for number of seedlings per subclass will give a unique value of the correlation coefficient, it is reasonable to expect that the two biserial distributions will give somewhat different values of the coefficient.

The weighting of the arrays of Table 3 as indicated above gives the two biserial distributions. (Tables 5 and 6.)

TABLE 5.—*Biserial interclass correlation table for number of seedlings per subclass (hill) in sea-island cotton, associated with alternative categories of Durango cotton of adjoining subplots (experiment 2/25, plot D–2–4)*

SEA-ISLAND

DURANGO	0	1	2	3	4	5	6	Total
S	389	194	232	389	587	933	771	3,495
D	1,651	226	218	301	433	567	309	3,705
Total	2,040	420	450	690	1,020	1,500	1,080	7,200

TABLE 6.—*Biserial interclass correlation table for number of seedlings per subclass (hill) in Durango cotton associated with alternative categories of sea-island cotton of adjoining subplots (experiment 2/25, plot D–2–4)*

DURANGO

SEA-ISLAND	0	1	2	3	4	5	6	Total
S	370	172	246	447	775	1,031	534	3,575
D	1,520	338	204	393	485	469	216	3,625
Total	1,890	510	450	840	1,260	1,500	750	7,200

The actual formation of the intraclass or interclass biserial table is unnecessary, since all that is required is ${}_s\bar{n}_s$ or ${}_d\bar{n}_s$, and $\bar{n}_s$, σ_{n_s}. Since ${}_s\sigma_{n_s}$, ${}_d\sigma_{n_s}$ may be desirable for other purposes, methods for their determination are given. Here the presubscript, s or d, denotes the character of the individual with which the weighted array of the value of n_s, upon which is based the mean or standard deviation, is associated. The absence of the presubscript indicates that $\bar{n}_s$, σ_{n_s} are based on arrays weighted with all individuals (both s and d) of the subclass with which they are associated.

CALCULATION OF BISERIAL INTRACLASS COEFFICIENTS FROM THE MOMENTS OF THE CLASSES

The moments for the array of the $(m-1)$ values of n_s associated with any s of any one of the m subclasses of the class are

$$\Sigma(n_s)-n_s' \quad \text{i}$$
$$\Sigma(n_s^2)-(n_s')^2 \quad \text{ii}$$

where Σ denotes the summation of all the values of n_s for the class and n_s' is the number of individuals of character s in the subclass with which the array of $(m-1)$ subclasses is associated.

Since there are n_s individuals of character s and $n-n_s=n_d$ individuals of character d in any subclass, i and ii must be weighted in an n_s-fold manner to obtain the moments for the weighted array associated with s, and in an $(n-n_s)$-fold, or in an n_d-fold, manner to obtain the moments for the weighted array associated with d for any subclass. Thus

$$n_s'\Sigma(n_s)-(n_s')^2 \quad \text{iii}$$

$$n_s'\Sigma(n_s^2)-(n_s')^3 \quad \text{iv}$$

are the moments for the weighted arrays associated with the fraction of individuals of any given subclass which have the character s, while

$$n_d'\Sigma(n_s) - n_d'\, n_s' = n\Sigma(n_s) - nn_s' - n_s'\Sigma(n_s) + (n_s')^2 \quad \text{v}$$

$$n_d'\Sigma(n_s^2) - n_d'\,(n_s')^2 = n\Sigma(n_s^2) - n(n_s')^2 - n_s'\Sigma(n_s^2) + (n_s')^3 \quad \text{vi}$$

are the moments for the weighted arrays associated with the fraction of individuals of any given subclass which have the character d.

Expressions iii and v give the first moments, while iv and vi give the second moments about 0 as origin for the weighted arrays associated with the individuals of any subclass of characters s (iii − iv) and d (v − vi), respectively.

But there are m such subclasses in any class and in consequence m values of iii − vi in any class.

Let S denote summation of the moments, weighted as indicated above, of the arrays associated with any subclass, and $\mathbf{S}$ denote summation for all the classes. For the whole sample iii and iv lead to

$$\mathbf{S}\{S[n_s'\Sigma(n_s)]\} - \mathbf{S}[S(n_s')^2] \quad \text{vii}$$

$$\mathbf{S}\{S[n_s'\Sigma(n_s^2)]\} - \mathbf{S}[S(n_s')^3] \quad \text{viii}$$

while v and vi lead to

$$\mathbf{S}\{S[n_d'\Sigma(n_s)]\} - \mathbf{S}[S(n_d'n_s')]$$
$$= \mathbf{S}\{S[n\Sigma(n_s)]\} - \mathbf{S}[S(nn_s')] - \mathbf{S}\{S[n_s'\Sigma(n_s)]\} + \mathbf{S}[S(n_s'^2)] \quad \text{ix}$$

$$\mathbf{S}\{S[n_d'\Sigma(n_s^2)]\} - \mathbf{S}[S(n_d'n_s'^2)]$$
$$= \mathbf{S}\{S[n\Sigma(n_s^2)]\} - \mathbf{S}[S(nn_s')] - \mathbf{S}\{S[n_s'\Sigma(n_s^2)]\} + \mathbf{S}[S(n_s')^3] \quad \text{x}$$

Since n_s' denotes merely any one of the m values of n_s, and S denotes summation for all values within one class, $S(n_s') = \Sigma(n_s)$, and $S\,[n_s'\Sigma(n_s)]$ may be written $[\Sigma(n_s)]^2$. Similarly $S[n_s'\Sigma(n_s^2)]$ becomes $\Sigma(n_s)\ \Sigma(n_s^2)$. These values are readily determined from frequency distributions of the number of individuals of character s per subclass.

Note further that for the class as a whole $S(n_s')^2 = \Sigma(n_s^2)$ and $S(n_s')^3 = \Sigma(n_s^3)$, and that $\mathbf{S}[\Sigma(n_s^2)]$ and $\mathbf{S}[\Sigma(n_s^3)]$ are merely the second and third moments about zero as origin of the values of n_s for the whole sample, and can easily be calculated from a frequency distribution of n_s.

Now remembering that weighting is in an $n(m-1)$-fold manner, the mean, ${}_s\bar{n}_s$, and the standard deviation, ${}_s\sigma_{n_s}$, of the values of n_s associated with individuals of character s may be written

$${}_s\bar{n}_s = \frac{\mathbf{S}\{[\Sigma(n_s)]^2\} - \mathbf{S}[\Sigma(n_s^2)]}{\mathbf{S}\{\mathrm{S}[n_s(m-1)]\}} \quad \text{xi}$$

$${}_s\sigma_{n_s} = \frac{\mathbf{S}[\Sigma(n_s)\ \Sigma(n_s^2)] - \mathbf{S}[\Sigma(n_s^3)]}{\mathbf{S}\{S[n_s(m-1)]\}} - {}_s\bar{n}_s^{\,2} \quad \text{xii}$$

In practical calculation it may be convenient to note that

$$\mathbf{S}\{S[n_s(m-1)]\} = [\Sigma(n_s)]\ (m-1)$$

Evaluation of these equations from the data in Table 1 gives, by way of illustration,

$$\mathbf{S}\{[\Sigma(n_s)]^2\} = 14{,}717;\ \mathbf{S}[\Sigma(n_s^2)] = 3{,}371$$

$$\mathbf{S}[\Sigma(n_s)\Sigma(n_s^2)] = 71{,}421;\ \mathbf{S}[\Sigma(n_s^3)] = 16{,}957$$

Whence, noting that $\mathbf{S}\{S[n_s(m-1)]\} = 2{,}860$; ${}_s\bar{n}_s = 3.967133$, ${}_s\sigma_s = 1{,}818024$, agreeing exactly with the constants calculated from the biserial distribution shown in Table 4.

The determination of ${}_d n_s$ and ${}_d\sigma_{n_s}$ proceeds in a similar manner

$${}_d\bar{n}_s = \frac{\mathbf{S}[\Sigma(n_d)\Sigma(n_s)] - \mathbf{S}[\Sigma(n_d n_s)]}{\mathbf{S}\{S[n_d\ (m-1)]\}} \qquad \text{xiii}$$

or substituting for n_d its equivalent $n - n_s$,

$${}_d\bar{n}_s = \frac{\mathbf{S}[\Sigma(n-n_s)\Sigma(n_s)] - \mathbf{S}\{\Sigma[(n-n_s)n_s]\}}{\mathbf{S}\{S[(n-n_s)(m-1)]\}}$$

$$= \frac{\mathbf{S}\{\Sigma(n)\Sigma(n_s) - [\Sigma(n_s)]^2\} - \mathbf{S}[\Sigma(nn_s) - \Sigma(n_s^2)]}{\mathbf{S}\{S[mn - mn_s - n + n_s]\}}$$

$$= \frac{\mathbf{S}[\Sigma(n)\Sigma(n_s)] - \mathbf{S}[\Sigma(nn_s)] - \mathbf{S}[\Sigma(n_s)]^2 + \mathbf{S}[\Sigma(n_s^2)]}{\mathbf{S}\{S[mn - mn_s - n + n_s]\}} \qquad \text{xiv}$$

If m and n both be constant, equation xiv may be written

$${}_d\bar{n}_s = \frac{\mathbf{S}\{n(m-1)[\Sigma n_s]\} - \mathbf{S}[\Sigma(n_s)]^2 + \mathbf{S}[\Sigma(n_s^2)]}{(m-1)\Sigma(n-n_s)} \qquad \text{xv}$$

$${}_d\sigma^2_{n_s} = \frac{\mathbf{S}[\Sigma(n_d)\Sigma(n_s^2)] - \mathbf{S}[\Sigma(n_d\ n_s^2)]}{\mathbf{S}\{S[n_d\ (m-1)]\}} - {}_d\bar{n}_s{}^2 \qquad \text{xvi}$$

or substituting for n_d its equivalent $n - n_s$,

$${}_d\sigma_{n_s} = \frac{\mathbf{S}[\Sigma(n-n_s)\Sigma(n_s^2)] - \mathbf{S}[\Sigma(n-n_s)n_s^2]}{\mathbf{S}\{S[(n-n_s)(m-1)]\}} - {}_d\bar{n}_s{}^2$$

$$= \frac{\mathbf{S}[\Sigma(n)\Sigma(n_s^2) - \Sigma(n_s)\Sigma(n_s^2)] - \mathbf{S}[\Sigma(nn_s^2)] - \Sigma(n_s^3)]}{\mathbf{S}\{S[mn - mn_s - n + n_s]\}} - {}_d\bar{n}_s{}^2$$

$$= \frac{\mathbf{S}[\Sigma(n)\Sigma(n_s^2)] - \mathbf{S}[\Sigma(nn_s^2)] - \mathbf{S}[\Sigma(n_s)\Sigma(n_s^2)] + \mathbf{S}[\Sigma(n_s^3)]}{\mathbf{S}\{S[mn - mn_s - n + n_s]\}} - {}_d\bar{n}_s{}^2 \qquad \text{xvii}$$

If m and n both be constant, equation xvii may be written

$${}_d\sigma_{n_s}{}^2 = \frac{\mathbf{S}\{n(m-1)[\Sigma(n_s^2)]\} - \mathbf{S}[\Sigma(n_s)\Sigma(n_s^2)] + \mathbf{S}[\Sigma(n_s^3)]}{(m-1)\Sigma(n-n_s)} - {}_d\bar{n}_s{}^2 \qquad \text{xviii}$$

The original data in Table 1 lead to

$$\mathbf{S}\{n(m-1)[\Sigma(n_s)]\} = 17{,}160 \qquad \mathbf{S}[n(m-1)\Sigma(n_s^2)] = 80{,}904$$
$$\mathbf{S}\{[\Sigma(n_s)]^2\} = 14{,}717 \qquad \mathbf{S}[\Sigma(n_s)\Sigma(n_s^2)] = 71{,}421$$
$$\mathbf{S}[\Sigma(n_s^2)] = 3{,}371 \qquad \mathbf{S}[\Sigma(n_s^3)] = 16{,}957$$

$$\mathbf{S}\{S[n_d(m-1)]\} = \mathbf{S}[(m-1)\Sigma(n-n_s)] = 2{,}900$$

and $${}_d\bar{n}_s = 2.004828, \quad {}_d\sigma_s = 2.257854$$

For the determination of the means and standard deviations of the whole population weighted as above indicated, the first and second moments on n_s may be obtained by adding the moments of ${}_sn_s$ and ${}_sn_d$. Thus

$$\bar{n}_s = \frac{\mathbf{S}\,[\Sigma\ (n)\ \Sigma\ (n_s) - \Sigma\ (nn_s)]}{\mathbf{S}\{S[n\ (m-1)]\}} \qquad \text{xix}$$

$$\sigma^2{}_{n_s} = \frac{\mathbf{S}\,[\Sigma\ (n)\ \Sigma\ (n_s)^2 - \Sigma\ (nn_s^2)]}{\mathbf{S}\{S\,[n\ (m-1)]\}} - \bar{n}_s^{\,2} \qquad \text{xx}$$

If m and n both be constant equations, xix and xx become

$$\bar{n}_s = \frac{\mathbf{S}\{[n\ (m-1)]\ [\Sigma\ (n_s)]\}}{(m-1)\ \Sigma\ (n)} \qquad \text{xxi}$$

$$\sigma^2{}_{n_s} = \frac{\mathbf{S}\{[n\ (m-1)]\ [\Sigma\ (n_s^2)]\}}{(m-1)\ \Sigma(n)} - \bar{n}_s^{\,2} \qquad \text{xxii}$$

For the present illustrative case the moments are already available and it is merely necessary to note that

$$(m-1)\ \Sigma\ (n) = \mathbf{S}\{S\,[n_s\ (m-1)]\} + \mathbf{S}[(m-1)\ \Sigma\ (n-n_s)] = 2{,}860 + 2{,}900 = 5{,}760$$

Thus $\bar{n}_s = 17160/5760 = 2.979167$

$$\sigma_{n_s} = [(80904/5760) - (2.979167)^2]^{1/2} = 2.273851$$

agreeing exactly with the constants derived from the row of totals of Table 4.

With these values in hand the calculation of the biserial intraclass coefficient proceeds by the method of Pearson (*13*).

The constants for n_s have been given above;

$${}_s\bar{n}_s = 3.967133$$
$$\bar{n}_s = 2.979167$$
$${}_s\bar{n}_s - \bar{n}_s = \bar{p} = +0.987966$$

Thus the mean number of seedlings per subclass is 0.99 higher in the subclasses associated with seeds of class s than in all the subclasses (hills) of the class $({}_s\bar{n}_s > \bar{n}_s)$. Clearly, therefore, there is a positive

correlation between the survival of a seed in one subclass and the number of seeds surviving in associated subclasses.

Now the probability of seeds germinating is $(2.979167)/6 = 0.496528$. Hence in the notation of Sheppard's tables (*17*), represented by Pearson (*16*)

$$\tfrac{1}{2}(1-\alpha) = 0.496528$$

$$\tfrac{1}{2}(1+\alpha) = 1 - \tfrac{1}{2}(1-\alpha) = 0.503472$$

For

$$\tfrac{1}{2}(1+\alpha) = 0.50 \text{ and } \tfrac{1}{2}(1+\alpha) = 0.51,\ \Delta = +0.003989 \text{ and } \Delta^2 = -0.000000$$

Hence

$$\Theta = (0.503472 - 0.500000)/0.003989 = 0.870393$$

$$\frac{\Theta(1-\Theta)}{2!} = \frac{0.870393 \times 0.129607}{2} = 0.056405$$

Determining z by the advancing difference formula

$$z = z_0 + \Theta\Delta_{z_0} - \frac{\Theta(1-\Theta)}{2!}\Delta_{z_0}^2 + \cdots$$

or numerically in the present case

$$z = 0.398942 - (0.870393 \times 0.000020) + (0.056405 \times 0.000040) = 0.398927$$

Finally, noting that for the whole series

$$\sigma_{n_s} = 2.273851$$

$$r = \frac{\bar{p}}{\sigma_{n_s}} \Big/ \frac{z}{\tfrac{1}{2}(1-\alpha)} = \frac{0.987960}{2.273851} \Big/ \frac{0.398927}{0.496528} = 0.5408$$

The intraclass biserial correlation coefficient derived by an identical procedure from the data for Durango cotton given in the right side of Table 1 is $r = 0.4284$.

CALCULATION OF BISERIAL INTERCLASS COEFFICIENTS FROM THE MOMENTS OF THE CLASSES

The moments for the μ values of ν_s associated with any one of the values of n_s of the associated class of m values of n_s are $\Sigma(\mu)$, $\Sigma(\mu^2)$. Weighting with the values of n_s, and denoting by S the summation for all m values of n_s and by $\mathbf{S}$ summation for all homologous classes

$$_s\bar{\nu}_s = \frac{\mathbf{S}\{S[n_s\Sigma(\nu_s)]\}}{\mathbf{S}[S(n_s\mu)]} = \frac{\mathbf{S}[S(n_s)\Sigma(\nu_s)]}{\mathbf{S}[\mu\Sigma(n_s)]}$$

$$_s\sigma^2{}_{\nu_s} = \frac{\mathbf{S}\{S[n_s\Sigma(\nu_s^2)]\}}{\mathbf{S}[S(n_s\mu)]} - {}_s\bar{\nu}_s^2 = \frac{\mathbf{S}[S(n_s)\Sigma(\nu_s^2)]}{\mathbf{S}[\mu\Sigma(n_s)]} - {}_s\bar{\nu}_s^2 \text{ .....xxiv}$$

Similar equations for ${}_d\bar{\nu}_s$, ${}_d\sigma_{\nu_s}$, $\bar{\nu}_s$, and σ_{ν_s} are easily written. Obviously the alternative classification may be based on the ν individuals of the subclasses of the classes of the second system, in which case the arrays required are those of ${}_sn_s$ and ${}_dn_s$ associated with individuals of class s and d of each value of ν, or the constants required are ${}_d\bar{n}_s$, ${}_s\sigma_{n_s}$, ${}_d\bar{n}_s$, ${}_d\sigma_s$, $\bar{n}_s$, σ_{n_s} all of which are readily obtained by a procedure similar to the above.

Two interclass coefficients may be obtained from the data of Table 1. Remembering that in this table n_s denotes number of seedlings per subclass (hill) in sea-island cotton, while ν_s denotes the comparable values in Durango cotton, the interclass correlation for the two arrays per hill of Durango cotton associated with the two alternative classes of sea-island cotton of the adjoining plot may be based on the moments

$$\mathbf{S}[S(n_s)\Sigma(\nu_s)] = 13{,}464 \quad \mathbf{S}[S(n)\Sigma(\nu_s)] = 20{,}970 \quad \mathbf{S}[S(n)\Sigma(\nu_s^2)] = 94{,}530$$

whence, noting that $\mathbf{S}[\mu\Sigma(n_s)] = 3{,}575$, and $\mathbf{S}[\mu\Sigma(n)] = 7{,}200$,

$${}_s\bar{\nu}_s = 3.766154 \quad \bar{\nu}_s = 2.912500 \quad \sigma_{\nu_s} = 2.155577$$

$$({}_s\bar{\nu}_s - \bar{\nu}_s) = (3.766154 - 2.912500) = +0.853654 = \bar{p}$$

The probability that the seeds of sea-island cotton with which the arrays of Durango cotton are associated will germinate and survive to the selected stage of development is, as in the preceding case (intraclass correlation, p. 288),

$$\frac{2.979167}{6} = \frac{3575}{7200} = 0.496528.$$

Hence, as before, $z = 0.398927$.

Finally

$$r = \frac{\bar{p}}{\sigma\nu_s} \Big/ \frac{z}{\frac{1}{2}(1-\alpha)} = \frac{0.853654}{2.155577} \Big/ \frac{0.398927}{0.496528} = +0.4929$$

Similarly, the interclass correlation for the two arrays per hill of sea-island cotton associated with the two alternative categories of Durango cotton of the adjoining plot may be based on the moments

$$\mathbf{S}[S(\nu_s)\Sigma(n_s)] = 13{,}464 \quad \mathbf{S}[S(\nu)\Sigma(n_s)] = 21{,}450 \quad \mathbf{S}[S(\nu)\Sigma(n_s^2)] = 101{,}130$$

whence, noting that $\mathbf{S}[m\Sigma(\nu_s)] = 3{,}495$ and $\mathbf{S}[m\Sigma(\nu)] = 7{,}200$,

$${}_s\bar{n}_s = 3.852361 \quad \bar{n}_s = 2.979167 \quad \sigma_{n_s} = 2.273851$$

$$({}_s\bar{n}_s - \bar{n}_s) = (3.852361 - 2.979167) = +0.873194 = \bar{p}$$

In determining the value of z it is necessary to base it on the alternative classes of Durango cotton with which the arrays of number of seedlings per subclass (hill) in sea-island cotton are associated. The probability of germination of Durango cotton is (p. 289)

$$\frac{\bar{\nu}s}{6} = \frac{2.912500}{6} = \frac{3495}{7200} = 0.485417 = \tfrac{1}{2}(1-\alpha)$$

Interpolating as before, from Sheppard's tables (*17*), $z=0.398676$. Finally

$$r=\frac{}{\sigma_{n_s}}/\frac{z}{\frac{1}{2}(1-a)}=\frac{0.873194}{2.273851}/\frac{0.398676}{0.485417}=+0.4676$$

The difference between the two coefficients, which can not be expected to be identical, is only 0.0253.

SUMMARY

Because of the inadequacy, or the innate limitations, of the available data, the biometrician frequently has to deal with variables classed in only two alternative categories. This has made necessary the development of correlation formulas suitable for dealing with (2×2)-fold, $(2\times n)$-fold, and $(3\times n)$-fold tables.

Such conditions are encountered in investigations of seedling mortality, in which it may be necessary to class individual seeds in only two alternative categories—"died" and "survived."

In an earlier paper (*10*) the applicability of Pearson's equivalent-probability correlation coefficient to the measurement of the intraclass correlations between the fate of the individual seedlings of the same hill has been indicated. Such coefficients are essentially intraclass coefficients (*4*), since the seedlings in each hill are regarded as forming a separate class (or subclass, as it is necessarily designated here).

The present paper has considered the problem of the extension of Pearson's biserial correlation method to intraclass and interclass distributions.

It has been shown that both intraclass and interclass biserial correlation coefficients may readily be determined from the moments calculated from the original data.

While illustrations of the application of the method to the problem of the influence of field heterogeneity, in the sense in which this term has been used in earlier papers (*5*, *6*, *7*, *11*, *12*), on seedling stand in sea-island and Durango cotton have been given, the primary purpose of the present investigation has been to derive the necessary biometric formulas. An extended application of the method to the problem of the influence of field heterogeneity on seedling stand in cotton will be discussed later.

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DEVELOPMENT OF BROWN CANKER OF ROSES [1]

By ANNA E. JENKINS

Associate Pathologist, Office of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The important rose disease known as brown canker, caused by *Diaporthe umbrina* Jenkins has been known in the United States for at least a quarter of a century.[2] [3] [4] The present known range of the disease includes most of the Atlantic Coast States, West Virginia, Kentucky, Pennsylvania, Alabama, Mississippi, Texas, and Michigan. Tea, hybrid tea, and hybrid perpetual roses are particularly susceptible. A few varieties in the following additional groups are also known to be affected: Pernetiana, Bengal or China, hybrid sweetbrier, dwarf polyantha, tea polyantha, Multiflora hybrid, Wichuriana hybrid, and Noisette hybrid. Rugosa, moss, and brier roses appear to be resistant or immune. Among the species on which the disease has been observed are the prairie (*Rosa setigera* Michx.), tea (*R. odorata* Sweet), dogbrier (*R. canina* L.), and cabbage (*R. centifolia* L.). The rose varieties on which the disease has been observed number about 175. Together with the rose species referred to above, these have already been listed.[5] [6]

This account presents data pertaining to the development of brown canker and its causal fungus on stems, blossoms, and leaves of roses grown in the rose test garden at the Arlington Experiment Farm of the Department of Agriculture near Rosslyn, Va., in the Department of Agriculture grounds at Washington, D. C., and in the Van Fleet collection at the United States Plant Field Station at Glenndale, Md., between the years 1921 and 1927. The data are supplementary to those previously published,[7] and are intended to aid in the diagnosis of brown canker, which is the first step in control.

STEM CANKER

Plate 1, A–C, shows initial infection lesions [8] on a plant of Gloire Lyonnaise on October 17, 1923. The first two stems represent the current year's growth and the third stem the growth of the preceding year. This was a control plant for a certain spraying experiment. Although search had been made, no evidence of initial infection had been found on current-year stems until early in August. At that time there were seen only two small lesions on a single stem; these were at first purple, later turning white. On one (pl. 1, A) of

[1] Received for publication Oct. 13, 1930; issued March, 1931.

[2] JENKINS, A. E. BROWN CANKER OF ROSES CAUSED BY DIAPORTHE UMBRINA. Jour. Agr. Research 15: 593–600, illus. 1918.

[3] ——— BROWN CANKER OF ROSES. Mycologia 17: 87–88. 1925.

[4] ——— BROWN CANKER OF THE ROSE. Amer. Rose Ann. 1927: 161–182, illus. 1927.

[5] JENKINS, A. E. Op. cit. (Excerpt of publication cited in footnote 4, pp. 182–183.)

[6] MARTIN, G. H., and JENKINS, A. E. PRELIMINARY LIST OF FUNGI AND DISEASES OF ROSES IN THE UNITED STATES. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 63: 354–369. 1928. [Mimeographed.]

[7] See footnotes 2, 3, and 4.

[8] Jenkins, A. E. Op. cit. (See footnote 4.)

the two stems representing current year's growth, purple spotting, or initial infection lesions, became visible about October 4 and on the other (pl. 1, B) approximately on September 10. In three weeks those on the second or larger stem had begun to turn white. The initial infection lesions on the older stem (pl. 1, C) were formed during the growing season of 1922 when that stem developed. Remaining quiescent during the succeeding season when advance from such lesions commonly takes place, they had as yet caused no appreciable injury to the stem. As determined by tissue cultures made from them, the fungus appeared to be as readily viable in these older lesions as in those on the current-year stems. In making the isolations small bits of diseased tissue were used as plantings. These were first dipped for about 30 seconds in a solution of mercuric chloride (1: 1,000) and then thoroughly rinsed in sterile water. Numerous plantings were made from each stem, some from lesions grouped above the leaf scars and others from those scattered about on the stems. The colonies were practically all pure for *Diaporthe umbrina*. (Fig. 2, C.) A few were of other fungi common on rose, namely, *Coniothyrium fuckelii* Sacc., *Gloeosporium* sp., and *Alternaria* sp. Pycnidia of *Coniothyrium fuckelii* have occasionally been found on whitish stem lesions which were apparently only those of brown canker. Whether this organism may also initiate such lesions has not been determined.

The illustrations in Plate 1, D–G, represent the rapid spring advance of cankers from initial infection lesions on 1-year-old stems of La Tosca (pl. 1, D) and of Gloire Lyonnaise (pl. 1, E and F) and from a larger canker on what is probably a 2-year-old stem (pl. 1, G). This material was gathered late in March, 1924. The lower as well as possibly the upper canker on the older stem apparently developed during the previous growing season. Here the fungus may have entered the stem through a blossom spur or a wound. The noticeable black specks in the newly formed cankers are pycnidia which have not yet broken through the epidermis. Conidia were already present in them. The fresh stems were placed in an iced refrigerator with their lower cut ends in water. After one week all of the cankers had advanced considerably. Conidia were then being exuded from the dark-colored pycnidia, which had ruptured the epidermis in the region of the lenticels. Perithecia containing immature asci and ascospores had also begun to form in the new cankers, but their presence could be detected only through microscopic examination.

The cankered stems shown in Figure 1 are representative of a condition which is often called "die-back." [9] When first observed on February 5, 1924, the stem of Col. R. S. Williamson (fig. 1, A) was entirely cankered to within 2 inches of a point near the base where earth had been mounded around it for winter protection. The fresh coloring of the diseased bark indicated that the die-back was fairly recent and that the fungus was then active. When photographed on March 17 the canker had advanced to, but not beneath, the surface of the soil; in some manner the earth may have acted as a barrier to its further downward progress. The La Tosca stem (fig. 1, B) is from a control plant employed in the spraying experi-

[9] LINZ, C. L. ROSE LOVERS AT THE NATION'S CAPITAL. Florists' Exch. 55: 1615, 1638. 1923.

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A-C.—Initial infection lesions of brown canker on current year (A and B) and 2-year-old (C) stems of Gloire Lyonnaise. × 1.

D-G.—Stems of La Tosca (D and G) and Gloire Lyonnaise (E and F), showing early spring advance of brown canker from initial infection lesions on growth of the previous year and from large cankers on that probably 2 years old (G). × 1.

(Color drawings by J. Marion Shull)

FIGURE 1.—Die-back due to brown canker on stems of (A) Col. R. S. Williamson, (B) La Tosca, and (C) Gloire Lyonnaise, collected in March (A and C) and in October (B). × 1

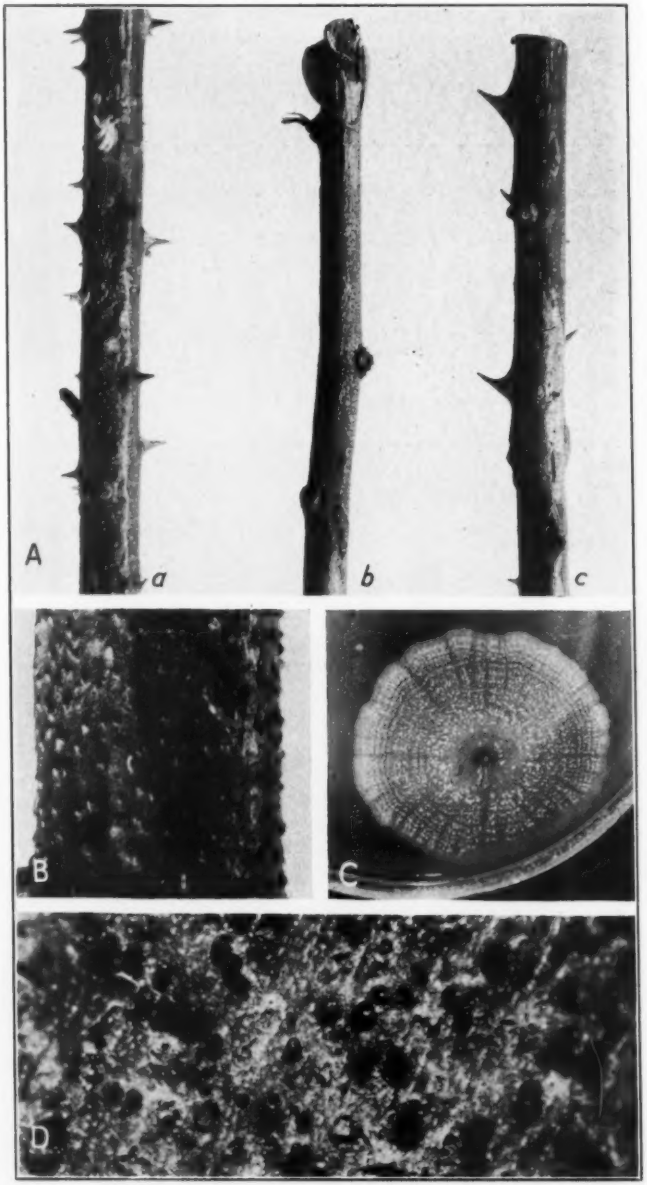

FIGURE 2.—A, Perithecial pustules of *Diaporthe umbrina* on rose stems collected in winter (*a*), summer (*b*), and autumn (*c*). × 1. B, Enlargement of part of canker shown in A, *b*. × 6. C, Sixteen-day-old isolation of *D. umbrina* on corn-meal agar medium in Petri dish, from tissue planting of initial infection lesions on stem shown in Plate 1, B. × 1. D, Pycnidia of *D. umbrina* on old dead stub of Killarney Queen. × 25

ment conducted at the Glenndale station during the growing season of 1926.[10] Collected on October 8, it illustrates die-back from blossom infection occurring during summer and autumn on current season's growth, such manifestation of brown canker having been practically controlled in the sprayed plants. Die-back on the

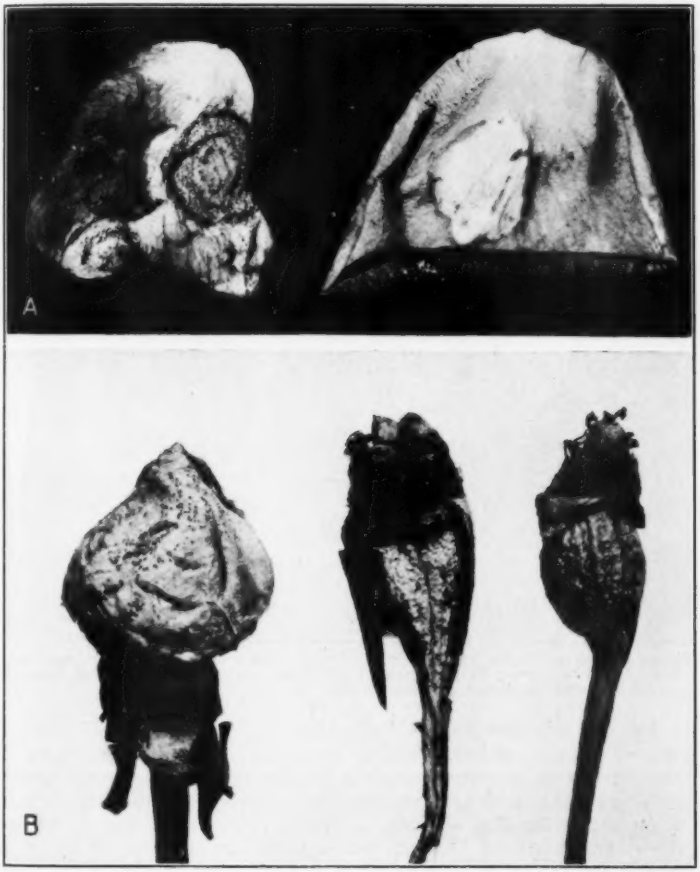

FIGURE 3.—A, Two fresh petals of Red Radiance infected by *Diaporthe umbrina*, on one of which are numerous pycnidia. × 1¼. B, Pycnidia of *D. umbrina* as present in June on unopened blossom and hips of a climbing hybrid tea rose which grew late the previous autumn. × 2

2-year-old stem of Gloire Lyonnaise (fig. 1, C) collected on March 25, 1924, occurred largely during the previous season.

The perfect stage of the fungus was present on all three of the cankered stems in Figure 2, A; part of the central stem is enlarged in Figure 2, B. The first stem, one of *Rosa corymbulosa*, was observed in January, 1924. It appeared to have been recently killed

[10] JENKINS, A. E. Op. cit. (See footnote 4.)

by the fungus, which was fruiting over practically its entire surface. The second and third stems were collected in June, 1924, and October, 1923, respectively. This material is illustrative of the fact that in the vicinity of Washington, D. C., the perfect stage of the fungus may be found during the autumn, winter, and summer, as well as during the spring of the year.[11] Old and unusually large pycnidia, each containing many conidia, are seen near the dead cut end of the stem of Killarney Queen shown in Figure 2, D. The falling away of the epidermis has left the pycnidia thus exposed.

BLOSSOM INFECTION

Fresh petals of Red Radiance affected by the brown-canker disease are shown in Figure 3, A. Infection is indicated in the first petal

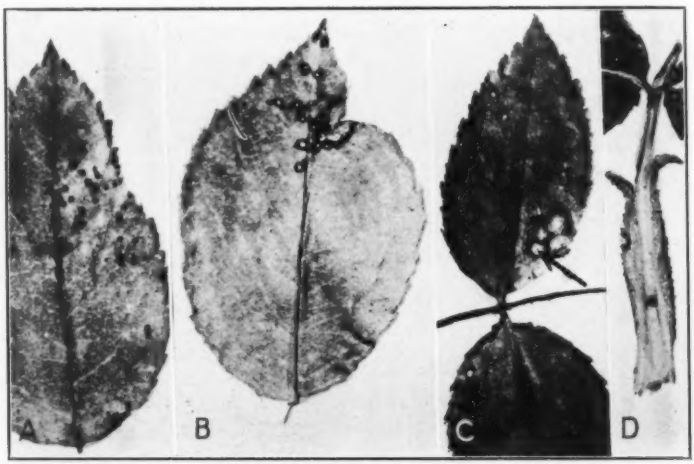

FIGURE 4.—Leaves of Gloire Lyonnaise and Oakmont showing leaf spot due to *Diaporthe umbrina:* A, Purple spotting; B, and C, larger or smaller lesions which are white or cinnamon-buff color at the center; D, petiole and stipule infection. × 1¾

only by the cinnamon-buff[12] discoloration characteristic of many lesions of brown canker, whether occurring on blossoms, stems, or leaves; in the other petal, pycnidia of the causal fungus are present in great numbers and are arranged in well-defined concentric circles, as often occurs in this species. (Fig. 2, C.) These petals, as well as the dead unopened blossom and two dead rose hips of a hybrid tea variety shown in Figure 3, B, were collected in June, 1921. The pycnidial stage of the fungus is also fruiting in abundance on the dead bud and hips, these having developed late the previous autumn.

INFECTION ON LEAVES

The leaf spot resulting from infection by *Diaporthe umbrina* is sometimes difficult to distinguish from other rose leaf spots. Recently formed purple spots or those that have remained purple are shown in Figure 4, A, and spots whose central regions have later

[11] JENKINS, A. E. Op. cit. (See footnote 4.)
[12] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

become white or cinnamon buff in Figure 4, B and C; a pycnidium has formed at the center of one of the small lesions. (Fig. 4, C, arrow.) Petiole and stipule infection are illustrated in Figure 4, D.

SUMMARY

This paper deals with the development of brown canker of the rose and its causal fungus, *Diaporthe umbrina*, on stems, blossoms, and leaves of roses grown in the vicinity of Washington, D. C. The data presented are supplementary to those previously published or are more detailed explanations of them. Several aspects of the disease and fungus are described.

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CONTROL OF MOISTURE CONTENT OF AIR AND WOOD IN FRESH-AIR CHAMBERS[1]

By IRA HATFIELD

Agent, Office of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture[2]

NEED FOR IMPROVED METHOD

Many pathological and physiological investigations call for a method of controlling the relative humidity of chambers in which experiments are to be conducted for a long period. Sulphuric acid solutions have been used extensively for such control, but the density of the solutions must be maintained definitely, and exchange of air in the chambers is greatly handicapped, if not entirely impeded. Criticism has also been made concerning the fumes that occur when this acid is used in the humidifiers. These fumes have received little attention as yet from those who have used the sulphuric-acid method.

In attempting to control the moisture content of wood blocks in an investigation of the moisture requirements of wood-inhabiting fungi, the writer needed an improved method of controlling the relative humidity. The features particularly desired were (1) a continuous supply of fresh air, (2) the maintenance of controlled relative humidity in culture chambers for indefinite periods of time, (3) freedom from contamination by microorganisms throughout the entire experiment, and (4) a means of collecting the respiratory products for use as an index of physiological activity.

SALT SOLUTIONS AND RELATIVE HUMIDITY

It is known that a saturated solution of a salt in contact with an excess of that salt will maintain a certain fixed relative humidity in an atmosphere confined above it, provided the temperature remains constant[3] and adequate air circulation is provided. Use was made of this fact in the present investigation through the selection of salt solutions having vapor tensions that would maintain the desired relative humidities.

A few of the salts used for this purpose are listed in column 1 of Table 1; column 2 gives the corresponding temperature ranges for the relative humidities as recorded in column 3, the values of which have been taken from the International Critical Tables.[4] A glance at column 3 reveals that there is enough difference in the relative humidity values for the salts to make a useful graded series. By using other salts listed in the critical tables a complete series from 2 per cent to 98 per cent humidity could be obtained.

[1] Received for publication Oct. 14, 1930; issued March, 1931.

[2] In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

[3] SPENCER, H. M. LABORATORY METHODS FOR MAINTAINING CONSTANT HUMIDITY. *In* International Critical Tables . . . 1:67-68. New York and London. 1926.

[4] SPENCER, H. M. Op. cit.

TABLE 1.—*Salts used for regulating the relative humidity in culture chambers and the moisture content of wood exposed to these relative humidities*

Salt	Temperature	Relative humidity	Equilibrium moisture content of southern yellow pine, based on oven-dry weight	
			Expected [a]	Found [b]
	°C.	Per cent	Per cent	Per cent
$Pb(NO_3)_2$	20	98	(c)	29.0
$NaBrO_3$	20	92	24.0	24.0
K_2CrO_4	20	88	21.7	21.7
KBr	20	84	19.9	19.7
$(NH_4)_2SO_4$	20–30	81 –81.1	18.7	18.7
NH_4Cl	20–30	79.2–79.5	18.1	18.0
$NaClO_3$	20	75	16.8	16.7
$NH_4Cl+KNO_3$ [d]	20–30	72.6–68.6	[e] 15.3	15.3
$Mg(C_2H_3O_2)_2 \cdot 4H_2O$	20	65	14.0	14.0
$NaBr \cdot 2H_2O$	20	58	12.5	12.4

[a] Obtained by averaging the two sets of values for southern yellow pine listed in Technical Note F–13 of the Forest Products Laboratory. This note also gives values for other species.
[b] Values obtained for *Pinus palustris* over a period of 14 weeks.
[c] Not given.
[d] Equal proportions of the 2 salts were used.
[e] At 70 per cent.

The usual temperature fluctuations in the laboratory in which this investigation was carried out are within the range between 20° to 30° C., inclusive. The table shows that it is possible to find salts that will give approximately the relative humidity values desired and at the same time maintain the individual values under usual laboratory temperature fluctuations. Further work on this phase of the problem is in progress.

APPARATUS FOR REGULATING RELATIVE HUMIDITY

The equilibrium moisture content of wood varies slightly from species to species. It seemed advisable, therefore, to check the moisture content definitely for a single species exposed to the specific conditions necessary in most pathological investigations. Accordingly, specimens of one of the southern yellow pines (*Pinus palustris* Miller) were placed in an apparatus based in principle upon that used by Heck [5] for soil work. Figure 1 presents a diagram of the apparatus. By means of an aspirator air is drawn through the intake tube containing soda lime. The chemicals remove the carbon dioxide and some of the water from the air. From this tube the air passes into a tower filled with pumice stone of such size as to permit its passage through a screen having four meshes to the inch. The stone has been treated with sufficient sulphuric acid to wet it thoroughly without allowing an excess of the acid to collect in the bottom of the tower. The sulphuric acid dries the air further and also aids in removing such laboratory gases as ammonia. The air then passes through another tower containing soda lime to assure that all the gases, including any acid fumes, have been removed. Any spores or organisms in the air are removed by conducting it through a U tube containing sterile cotton. The air is then bubbled through two Erlenmeyer flasks containing the salt solutions chosen to give the desired

[5] HECK, A. F. A METHOD FOR THE DETERMINATION OF TOTAL CARBON AND ALSO FOR THE ESTIMATION OF CARBON DIOXIDE EVOLVED FROM SOILS. Soil Sci. 28: 225–233. 1929.

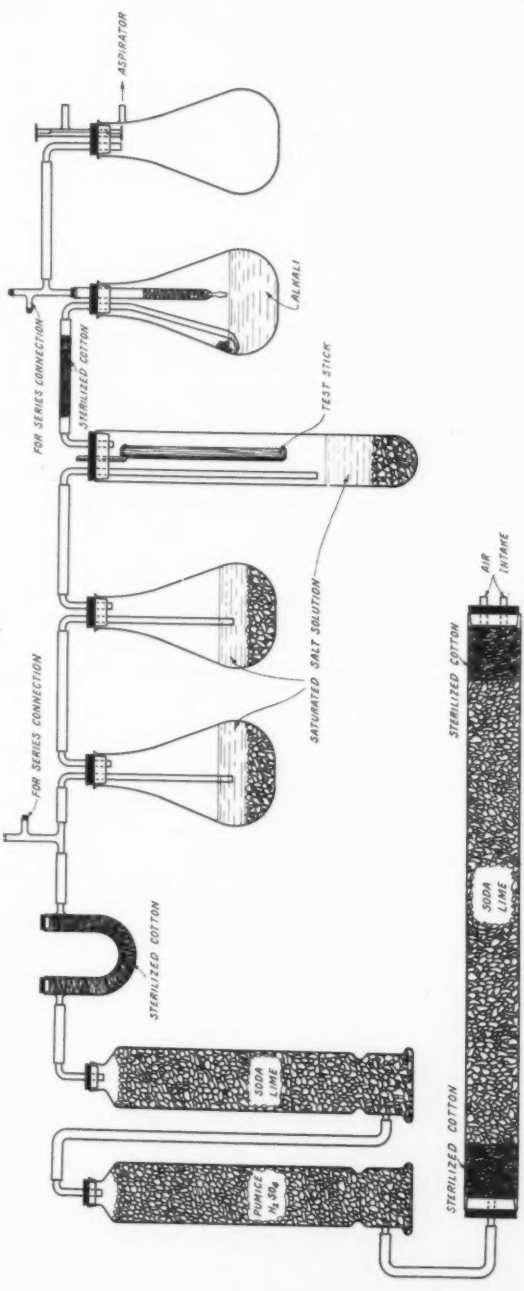

FIGURE 1.—Apparatus for regulating relative humidity

relative humidity. This brings it to the proper moisture content. In the culture chamber is more of the salt solution, to aid in maintaining the proper relative humidity. In this chamber the air is not bubbled into the solution, but is drawn in close to the surface of the liquid, which prevents spraying of the solution on to the test stick. The test stick is suspended on a bent glass rod, thus avoiding the use of a corrodible metal hanger.

The air from the culture chamber is conducted into a carbon-dioxide trap constructed as described by Heck [6] and as represented in Figure 1. As the air is drawn from the culture chamber it passes through a rubber-tubing connection that is filled with cotton. The cotton in the tubing serves only as a means of keeping contamination from entering the culture chamber when the carbon-dioxide trap is removed for titration purposes. From the cotton-filled tubing the air passes through the entrance tube to the carbon-dioxide trap. This entrance tube is drawn out at its lower end and bent toward the side of the flask to make the bubbles adhere to the side of the flask, thus affording greater diffusion of carbon dioxide into the water before the bubbles finally burst. The exit of air from the trap is made through a bead tower, where any remaining traces of carbon dioxide can be absorbed by the capillary film of alkaline solution around each bead. The bead tower is made by drawing out a ¾-inch glass tube and so flattening the constriction that two beads will lie at the bottom of it, with enough space remaining to allow a free passage of fluid either way. This facilitates an easy and complete washing of the tower and the solid glass beads when titrations are to be made. The use of this carbon-dioxide trap makes possible the collection of the carbon dioxide given off by any fungus that may be growing on or in the test stick. Measurement of the carbon dioxide by titration gives curves that may be used as an indication of the growth of the fungus at the given moisture content.

The chief purpose of the final flask shown in Figure 1 is to permit the attachment of a vacuum gauge and an aspirator. Between this flask and the U tube are the four chambers last described, and on each side of the four is a T connection, shown with its free ends closed. A series of groups of chambers, four in each group, may be connected between these T's. Thus a whole battery of culture chambers can be kept at graduated humidities with air drawn from the same towers. For different humidities it is necessary to duplicate only the part of the apparatus shown between the points marked "for series connections" in Figure 1.

When several tests are set up in parallel the rate of air movement through the chambers can be distributed evenly by means of stopcocks applied either at the rubber connection previous to the first Erlenmeyer flask containing the saturated salt solution, or at the rubber connections between the carbon-dioxide titration chamber and the tube leading to the aspirator. Uniform rapid air exchange is desirable, as it keeps the moisture gradient of the stick more nearly uniform and assures complete removal of carbon dioxide as fast as it is produced.

Column 5 of Table 1 shows the fine gradient obtained from the various salts when they were used to control the moisture content of

[6] HECK, A. F. Op. cit.

the test sticks placed in the various culture chambers in a consecutive series of humidities. The experimental results for the one species examined agree very well with the equilibrium moisture content values for southern yellow pine presented in Technical Note F–13 of the Forest Products Laboratory [7] and as averaged by means of curves and tabulated in column 4 of Table 1. This agreement means that the test sticks dried down to the equilibrium moisture content expected for each value of relative humidity, and were maintained at that moisture content for several months within satisfactorily small limits of experimental error. It was necessary, of course, to keep each test stick in its culture chamber long enough to insure the attainment of equilibrium with the moisture conditions of the atmosphere surrounding it. Mention should also be made of the fact that certain salts, otherwise satisfactory for controlling humidity, can not be used when carbon-dioxide tests are to be made.

Although the apparatus described was designed especially for studies of the growth of wood-destroying fungi, it could easily be adapted for spore-germination or seed-germination tests, the culture of small plants, and perhaps for other purposes. Where experiments with Petri-dish cultures or other cultures are to be made, it would be necessary only to modify the culture chamber diagramed in Figure 1. This modified chamber might take the form of a special vacuum desiccator or a culture chamber similar to the one used by Fellows [8] for determining the influence of oxygen and carbon dioxide on the growth of *Ophiobolus graminis* in pure culture.

[7] UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST SERVICE, FOREST PRODUCTS LABORATORY. MOISTURE CONTENT OF WOOD AT DIFFERENT HUMIDITIES. U. S. Dept. Agr., Forest Serv., Forest Prod. Lab. Tech. Note F–13, 2 p. 1919. [Mimeographed.]

[8] FELLOWS, H. THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF OPHIOBOLUS GRAMINIS IN PURE CULTURE. Jour. Agr. Research 37: 349–355, illus. 1928.

INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN HYBRIDS OF WHITE FEDERATION AND BANNER BERKELEY WHEATS[1]

By FRED N. BRIGGS[2]

Associate Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and *Associate in Agronomy, California Agricultural Experiment Station*

INTRODUCTION

In previous papers (*1, 3, 4*)[3] the inheritance of resistance to bunt, *Tilletia tritici,* (Bjerk.) Wint., in hybrids between susceptible varieties of wheat and the resistant varieties Martin, Hussar, and White Odessa has been reported. It was shown that Martin differs from such susceptible varieties as White Federation in one main dominant factor for resistance to this disease (*1*). Hussar differs from susceptible varieties in two factors, one of which is the same as the dominat factor in Martin, but the other allows bunt to develop in about half the heterozygous plants (*1, 3*). White Odessa has a single dominant factor for resistance which is similar in its effect to the Martin factor (*4*). Whether this factor is identical with the Martin factor has not been determined definitely.

The present paper is concerned with studies on the inheritance of resistance to bunt in a hybrid between susceptible White Federation and resistant Banner Berkeley.

METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. Conditions there favor such investigations because relatively high bunt infection can be obtained when wheat is sown in the fall. Both spring and winter varieties may be seeded at that time without any danger of winterkilling and with the assurance that both types will mature the following summer.

The seeds were thoroughly blackened with bunt spores. The inoculum was collected by W. W. Mackie in 1917 on Little Club wheat in the Montezuma Hills district of Solano County, Calif. It was propagated by Mackie on Little Club wheat in the botany garden at Berkeley, Calif. Since 1919 the writer has grown smut from this original collection on White Federation wheat at Davis. The inoculum used, therefore, has been derived from one original collection of bunt. Since Faris (*6*) showed that physiologic forms of bunt exist, the writer has been especially careful not to introduce new collections of bunt. The fact that the same collection of bunt has been used continuously at Davis makes it reasonably certain that the same form or mixture of forms has been employed in all these investigations. This

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[2] The writer acknowledges valuable suggestions from Dr. R. E. Clausen, division of genetics, and various members of the division of agronomy, University of California, and from various members of the Office of Cereal Crops and Diseases.

[3] Reference is made by number (italic) to Literature Cited, p. 313.

is indicated also by the consistent way in which the parent wheat varieties have reacted to this inoculum.

The wheat seeds were spaced from 2 to 3 inches apart in rod rows 1 foot apart. The entire nursery was sown within three or four days, in order to avoid the effects of different temperatures and soil moistures.

At harvest the plants in each row were pulled and separated into two piles, one bunt free and the other bunted. The total number of plants and the number of bunted plants were recorded, and the percentage of bunt infection was calculated. A plant was classified as bunted if it showed any infection.

According to Tisdale et al. (*9*), Banner Berkeley produced an average of 1.1 per cent of bunt at Moro, Oreg., in the three years 1920 to 1922, but produced no bunt at Pullman, Wash., in 1922. The writer obtained a supply of seed from H. M. Woolman in the fall of 1921, and this variety had 3.2 per cent of bunt in 1922 at Davis, Calif. Banner Berkeley was not grown in the smut nursery again until 1927, but has been bunt free since that time. From 1922 to 1926 it was propagated in the parent nursery by growing a row each year from a single head. In 1926 one plant was crossed with White Federation and Martin wheats. The stock of seed used since that time has been propagated from that plant.

Just why Banner Berkeley produced a little bunt in 1922 and has been bunt free since 1927 under similar conditions when inoculated with bunt from the same original source is not known. A similar condition was encountered with White Odessa wheat. It is possible that the original lot of seed was not a pure line. The original collection of bunt may have consisted of two or more physiologic forms of bunt, one of which could attack Banner Berkeley slightly. The latter form could have been lost in propagating the inoculum on White Federation. Dillon-Weston (*5*) has shown that it is possible to isolate more than one physiologic form from a single collection of bunt. Finally, the seed of Banner Berkeley as obtained from Mr. Woolman may have been slightly contaminated with a form of bunt which could attack it. It is known now that such forms exist in Oregon.

That White Federation is very susceptible as compared with Banner Berkeley may be seen in Table 1.

TABLE 1.—*Annual percentage of bunt infection in the parent wheat varieties in four years, 1922 and 1927-1929, when grown at Davis, Calif.*

Variety	Percentage of bunted plants				
	1922	1927	1928	1929	Average
Banner Berkeley	3.2	0.	0.	0.	0.8
White Federation	58.3	66.6	68.9	78.6	68.1

EXPERIMENTAL RESULTS

The crosses White Federation × Banner Berkeley and Martin × Banner Berkeley were made in 1926. The latter cross was made to determine whether the Martin factor was present in Banner Berkeley. The F_1 seeds were not inoculated because of the small number available.

A part of the F_2 seeds of both crosses were treated with copper carbonate to protect them from bunt infection, in order to grow a supply for F_3. Enough seeds of each cross to plant 20 rod rows were inoculated and the F_2 was grown in 1928. Twenty-two additional F_2 rod rows of White Federation × Banner Berkeley were grown in 1929.

The F_2 data do not permit a satisfactory Mendelian analysis, because some susceptible plants usually escape infection. Besides, resistant and heterozygous plants occasionally may become infected. They do indicate the number of factors present and also the percentage of bunted plants to be expected in F_3 rows of the same genotype. Data collected in F_2 are recorded in Table 2.

TABLE 2.—*Percentage of bunted plants in the parents and the F_2 of the crosses named when grown in the field at University Farm, Davis, Calif.*

Parent or cross	Year grown	Number of plants		Percentage of bunted plants
		Total	Bunted	
Banner Berkeley	1927	151	0	0
Do	1928	491	0	0
Do	1929	532	0	0
White Federation	1928	309	253	81.9
Do	1929	492	428	87
Martin	1928	293	0	0
Do	1929	232	0	0
F_2 Martin×Banner Berkeley	1928	893	0	0
F_2 White Federation×Banner Berkeley	1928	965	230	23.8
Do	1929	1,034	244	23.6

There was an average of 23.6 per cent of bunted plants in the F_2 of White Federation × Banner Berkeley, which is very near the 25 per cent expected on the basis of a single dominant factor for resistance. Martin × White Federation produced 17.2 per cent of bunted plants in F_2 (*1*). At that time it was shown that enough susceptible plants had escaped infection to bring this figure into satisfactory agreement with the 25 per cent expected. White Odessa × White Federation had 22.3 per cent of bunted plants in F_2 (*4*). In the case of White Federation × Banner Berkeley, as with the White Odessa cross, either fewer susceptible plants escaped infection or more resistant and heterozygous plants became infected. That resistant and heterozygous plants occasionally may become infected has been shown in other papers (*2*, *4*). The F_2 data, then, indicate that Banner Berkeley differs from White Federation in a single dominant factor for resistance to bunt, and that heterozygous F_3 rows should contain an average of about 24 per cent of bunted plants.

F_2 of Martin × Banner Berkeley was free from bunt, indicating that Banner Berkeley has the same factor for resistance as Martin. This point is confirmed by F_3 data, which will be presented later.

In F_3, 301 rod rows were grown from 301 F_2 plants which had been protected from bunt infection by seed treatment. Obviously the classification of F_2 plants on the basis of the behavior of their progeny in F_3 rows is more reliable than classification in F_2. F_3 rows contained from 30 to 60 plants. F_3 data are recorded in Table 3.

TABLE 3.—*Distribution of the parent and the F_3 rows of the crosses named into 5 per cent classes for bunt infection, when grown at Davis, Calif., in 1929*

Parent or cross	Distribution of rows by percentage classes of bunt infection																					Total number of rows
	0	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	
White Federation																		4	3	2	1	10
Martin	5																					5
Banner Berkeley	12																					12
White Federation × Banner Berkeley	57	10	4	15	32	59	29	19	5	3	1	0	2	1	0	3	12	13	10	16	10	301
Martin × Banner Berkeley	100																					100

The rows in the 0–5 per cent class were separated into those with no bunted plants and those with 1 to 5 per cent of bunted plants because of the special interest in the former. The nature of the distribution may be seen more readily in Figure 1.

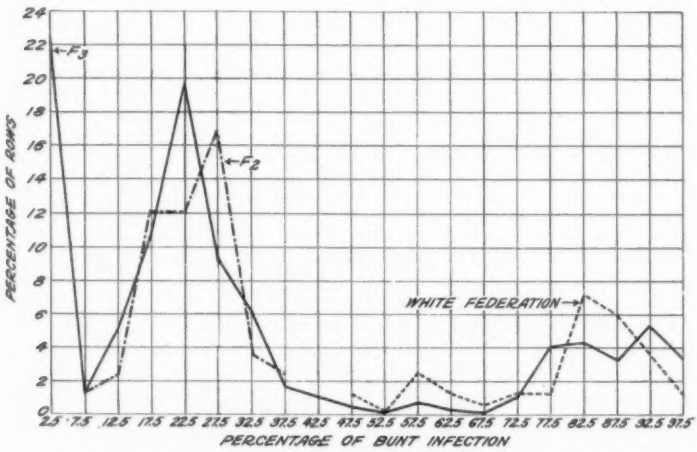

FIGURE 1.—Distribution of F_2 and F_3 rows of the cross White Federation × Banner Berkeley and rows of the White Federation parent into 5 per cent classes for bunt infection. (The solid line represents 301 F_2 rows, the dot-and-dash line 42 F_2 rows, and the broken line 42 rows of White Federation)

The numbers of rows under the three modes agree satisfactorily with the 1:2:1 ratio. There were 69 resistant rows where 75.25 were expected, 165 heterozygous rows where 150.5 were expected, and 67 susceptible rows where 75.25 were expected. The minima should not be thought of as marking with absolute accuracy the divisions between phenotypes. However, the first minimum in the crosses with Martin, White Odessa, and Banner Berkeley fell at 7.5 per cent of bunt. In the same crosses the second minimum fell very near 50 per cent. The numbers of rows in this region are so few that any point from 40 to 60 would not change materially the numbers of rows under the adjacent modes.

The 69 resistant rows were made up of 57 which were completely resistant and 12 which had from 1 to 7.5 per cent of bunted plants.

Data have been published (*2*) which show that this percentage of bunt may be due to modifying factors and that these rows do belong in the resistant group.

There were 67 susceptible rows, which contained from 52.5 to 97.5 per cent of bunted plants. The 10 rows of White Federation which were grown as checks among the 301 F_3 rows of White Federation × Banner Berkeley had from 82.5 to 97.5 per cent of bunt. These did not approach the lower limits of bunt found in susceptible F_3 rows, but the number of parent rows was small. In the entire smut nursery there were 42 rows of White Federation, which had from 47.5 to 97.5 per cent of bunted plants. These are shown in Figure 1 as a broken line. The distribution of the 42 rows of White Federation agrees very well with the distribution of susceptible F_3 rows. The writer usually plants in paired rows. The two rows commonly differ in bunt infection by 5 to 10 per cent. In one case adjacent rows of the above lot differed by 30 per cent, indicating that environmental conditions may change radically from row to row.

There were 165 heterozygous rows with an average of 24.1 per cent of bunted plants. Twenty F_2 rows produced an average of 23.8 per cent of bunted plants in 1928, and 22 F_2 rows averaged 23.6 per cent in 1929. The 42 F_2 rows are plotted with a dot-and-dash line over the heterozygous group of F_3 rows in Figure 1. The two curves agree very well in essential features.

TABLE 4.—*Distribution of F_4 rows from certain F_3 rows of the cross White Odessa × White Federation into 5 per cent classes for bunt infection, when grown at Davis, Calif., in 1929*

Distribution of rows by percentage classes of bunt infection	F_4 rows from F_3 row No. 1092 with 13.1 per cent of bunt		F_4 rows from F_3 row No. 1347 with 14.6 per cent of bunt		F_4 rows from F_3 row No. 1349 with 31.7 per cent of bunt		F_4 rows from F_3 row No. 1360 with 36.7 per cent of bunt	
	Classes	Modes	Classes	Modes	Classes	Modes	Classes	Modes
0	11	15	13	14	11	12	2	6
1–5	3		2		1		3	
5–10	2		5		1		1	
10–15	10		9		3		1	
15–20	9		2		8		1	
20–25	8	37	7	28	7	26	1	17
25–30	3		4		3		2	
30–35	4				2		3	
35–40	2				1		6	
40–45					1		2	
45–50	1	[a] 9	1	[b] 7		[d] 15	1	[c] 6
50–55	1							
55–60	1				1			
60–65	1							
65–70	2				1			
70–75					1			
75–80								
80–85					1			
Total rows	61		49		53		29	
P	0.122		0.234		0.801		0.654	

[a] Includes 3 plants that were completely smutted in F_3 and therefore produced no good seed.
[b] Includes 6 plants that were completely smutted in F_3 and therefore produced no good seed.
[c] Includes 5 plants that were completely smutted in F_3 and therefore produced no good seed.
[d] Includes 11 plants that were completely smutted in F_3 and therefore produced no good seed.

Obviously it is not practicable to check the accuracy of the classification of very many F_3 rows by growing the F_4. Four F_3 rows of the heterozygous group from White Odessa × White Federation (*4*) now

have been checked in F_4. Two rows were selected from near the lower limits and two rows from near the upper limits for bunt infection in the heterozygous group. These rows had, respectively, 13.1, 14.6, 31.7, and 36.7 per cent of bunted plants. The extreme limits of the heterozygous group were avoided, because there is no reason to believe that there is not some overlapping. It therefore would have been desirable to check a larger number of F_3 rows, which was not practicable. That the four rows described belong to the heterozygous group may be seen from Table 4.

If the plants that were completely smutted in F_3 are classed as susceptible and are added to the F_4 rows that are classed as susceptible, the agreement with the 1:2:1 ratio in all four families is satisfactory. Table III by Fisher (*7*) was used in calculating the *P* values. These four F_3 rows were properly classified as heterozygous.

The classification of F_2 plants, determined on the basis of the percentages of bunted plants in F_3 rows, shows that Banner Berkeley differs from White Federation in one main dominant factor for resistance to bunt. That this factor is identical with the factor in Martin is evidenced by the fact that there was no bunt in the F_2 or 100 F_3 rows of Martin × Banner Berkeley.

DISCUSSION AND SUMMARY

The literature pertinent to the inheritance of resistance to bunt has been reviewed and discussed in earlier publications and will not be repeated here.

The writer has presented data (*1*) which show that the Martin variety differs from such susceptible wheats as White Federation in one main dominant factor for resistance to bunt. Hussar wheat (*1*, *3*) differs from such susceptible varieties in two factors for resistance to this disease. One is the same as the factor present in Martin, but the other allows bunt to develop on about half of the heterozygous plants. Martin may be represented by *MMhh* and Hussar by *MMHH*. White Odessa wheat (*4*) has a single factor for resistance to bunt which is similar in its effect to the factor present in Martin. Whether or not it is identical with the Martin factor is not known at present. The data now available suggest that they are identical and that White Odessa should be designated as *MMhh*. Data presented in this paper show that Banner Berkeley differs from White Federation in one main factor for resistance to bunt. This factor is identical with the one present in Martin, therefore Banner Berkeley is of the *MMhh* constitution.

The bunt used in all these experiments is the one designated as physiologic race III of *Tilletia tritici* by Reed (*8*). There may be other factors for resistance to bunt in Martin, Hussar, White Odessa, and Banner Berkeley which would have become apparent in the presence of other physiologic forms of bunt. If there are no other factors present, Martin (*MMhh*), Banner Berkeley (*MMhh*), and White Odessa, if the *MMhh* designation is correct, should react the same to all physiologic forms of bunt. Furthermore, Hussar (*MMHH*) should not be susceptible to any forms to which the Martin group is resistant. However, Hussar should be resistant to some forms which attack Martin, because of the *H* factor. There are not enough data available at present to verify this point.

The study of physiologic forms of bunt has been complicated somewhat because monosporic inoculations have not been possible with this disease. The situation will be improved wherever it is possible to harbor a particular physiologic form of bunt on a variety that is resistant to all other forms that are being investigated. That field collections of bunt may not be pure physiologic forms has been shown by Dillon-Weston (*5*). He was able to isolate a very virulent form from a relatively nonvirulent collection of inoculum when used on Sherman wheat. Undoubtedly he was dealing with a mixture of forms in which the virulent form was present in relatively small amounts.

In breeding bunt-resistant varieties of wheat it is immaterial, as far as bunt resistance is concerned, whether Martin or Banner Berkeley is used for the resistant parent, because they both have the *MMhh* constitution for resistance.

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